REVIEWS: CURRENT TOPICS

The Nrf2-antioxidant response element pathway: a target for regulating energy metabolism ☆

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Abstract

The nuclear factor E2-related factor 2 (Nrf2) is a transcription factor that responds to oxidative stress by binding to the antioxidant response element (ARE) in the promoter of genes coding for antioxidant enzymes like NAD(P)H:quinone oxidoreductase 1 and proteins for glutathione synthesis. The Nrf2/ARE pathway has nutritional interest owing to its activation by phytochemicals such as sulforaphane. Recently, the Nrf2 pathway was identified as having regulatory functions in mitochondrial biogenesis, adipocyte differentiation and liver energy metabolism. Activation of Nrf2 increases energy metabolism and conversely suppresses lipid synthesis. Lard-based, but not soybean oil-based, high-fat diets reduce mRNA expression of Nrf2 and its downstream targets, suggesting a macronutrient influence on the activation of the Nrf2 pathway and susceptibility to oxidative stress. This review examines data revealing the Nrf2 pathway's regulatory role in energy metabolism at the molecular, cellular and whole animal levels. Understanding the relationship of Nrf2 and energy metabolism in cells, tissues and physiologic systems will provide novel insights for nutritional interventions for obesity and its comorbidities such as diabetes.
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1. Introduction

Given the alarming epidemic of obesity and its related diseases worldwide, there is a renewed focus upon elucidating the mechanisms underlying the regulation of energy metabolism, mitochondrial function and control of cellular redox balance. The relationship of energy metabolism, redox chemistry and generation of reactive oxygen species (ROS) is easily apparent at the mitochondrial level in which superoxide is generated as a consequence of the electron transport chain activity and Kreb's cycle activity. Exciting, new data indicate that nutrient intake, energy metabolism and ROS are also linked at the nuclear level via the nuclear factor E2-related factor 2 (Nrf2)/antioxidant response element (ARE) pathway.

Nrf2 is commonly recognized to augment cellular defense against elevated oxidative damage. At its simplest, the Nrf2 transcription factor resides in cytoplasm until increases in cellular oxidative stress cause it to translocate to the nucleus. Nrf2 binds to the cis-acting enhancer ARE sequence (core sequence: TGAG/CNNNGC) present in promoters of genes that code for proteins necessary for glutathione synthesis and electrophile detoxification [1]. Transcriptional activation of ARE-mediated genes requires that Nrf2 heterodimerizes to other basic leucine zipper proteins, including Jun (c-Jun, Jun-D and Jun-B) and small Maf (MafG, MafK and MafF) [2,3]. Additionally, the nrf2 gene contains two ARE-like sequences in its promoter so that Nrf2 can autoregulate itself and potentially sustain ARE-mediated gene expression longer [3,4]. Newer data detailed below suggest that Nrf2 indirectly affects gene expression through inhibition of the p300 acetylase [5].

Recent research shows that the Nrf2/ARE pathway plays an important role at the intersection of energy metabolism and macronutrient intake. Genomic, proteomic and physiologic data from several laboratories implicate Nrf2 in energy metabolism and may be a target for treatment of obesity-related disorders. In this work, we will review the current data detailing the role of the Nrf2/ARE pathway as it relates to energy metabolism and energy storage, as well as identify areas for further research.

2. Regulation of Nrf2

Our knowledge of the cytoplasmic and nuclear disposition of Nrf2 has greatly increased in recent years and is necessary for understanding the context of Nrf2 in energy metabolism. Enhancement of Nrf2 pathway activity can occur through interrupting the cytoplasmic degradation of Nrf2. Under homeostatic conditions, Nrf2 pathway activity is suppressed by sequestration and degradation of Nrf2 in the

cytoplasm through an interaction with the actin-bound protein Keap1 (Fig. 1). The Neh2 domain (amino acid residues 1–95) of Nrf2 binds with the region between the BTB and Kelch repeat domains of Keap1. This binding then serves as an adaptor for the cullin3/ring box 1 (Cul3/Rbx1) E3 ubiquitin ligase complex, which elicits the ubiquitination of Nrf2 that then is degraded by the 26S proteasome [2,6,7]. Furthermore, Keap1 will sequester Nrf2 to the mitochondria through a ternary complex including the phosphoglycerate mutase protein PGAM5 (Fig. 1) [8,9]. This sequestration of Nrf2 to the mitochondria is suggested to allow for enhanced Nrf2 activation in response to mitochondrial stressors [9].

Within the BTB/Kelch repeat region, there are 4 out of 25 available cysteine residues (Cys257, Cys273, Cys288 and Cys297) that function as sensors for inducer ligands or oxidative stress within the cell's environment. When these cysteines are oxidized by ROS or lipid oxidation products, Keap1 undergoes conformational changes and release of Nrf2 [2,10,11]. Similarly, phosphorylation of serine 40 on Nrf2 by protein kinase C also leads to the dissociation of Nrf2 from Keap1 [12]. Blocking this interaction leads to subsequent translocation of Nrf2 to the nucleus where it is able to enhance or blunt the expression of multiple genes [2,5,13–16].

Nrf2 activity is also regulated by phosphorylation via activity of glycogen synthase kinase-3β (GSK-3β), an enzyme whose activity is blocked by insulin signaling (Fig. 1) [17]. GSK-3β is constitutively active within a cell until an extracellular signal, such as insulin receptor activation, inhibits it. Elevated insulin signaling, in response to feeding, activates protein kinase B (PKB)/Akt via phosphorylation by phosphoinositide-dependent kinase 1/2. In turn PKB/Akt inhibits GSK-3β through phosphorylation of Ser9 on GSK-3β [17]. Initial work by Salazar and colleagues demonstrated that GSK-3β inhibits Nrf2 activity via phosphorylation of 10 potential serine residues in Nrf2 [15]. They further determined that GSK-3β controls the subcellular distribution of Nrf2 through sequestration in the cytoplasm [15]. Rada and colleagues additionally elaborated this mechanism, showing that GSK-3β phosphorylates a cluster of serine residues (Ser335, Ser338, Ser342, Ser347, Ser351 and Ser355) within the Neh6 domain of Nrf2 [18]. These serine residues within the Neh6 domain overlap a destruction motif recognized by the SCF/β-TrCP E3 ubiquitin ligase. The SCF/β-TrCP protein binds and recruits a scaffold protein, Cullin-1, to this destruction motif which then allows for linker proteins Skp1 and Rbx1 to associate and form a complete ubiquitin E3 ligase [18]. Nrf2 thus becomes ubiquinated by the ligase complex and is targeted for degradation. Rada and colleagues also showed that the SCF/β-TrCP complex works in concert with the canonical Keap1-independent degradation pathway, therefore leading to a proposed "dual degradation" model between the GSK-3β–SCF/β-TrCP complex and Keap1 for regulating Nrf2 activity [18].

In an alternative mechanism involving GSK-3β, Jain and Jaiswal determined that Nrf2 is not directly phosphorylated by GSK-3β but, instead, GSK-3β phosphorylates the threonine residues of Fyn kinase [19]. Fyn then translocates to the nucleus and directly phosphorylates tyrosine 568 of Nrf2. This leads to the export, ubiquitination and degradation of Nrf2. After discovering Fyn's role in regulating Nrf2, Jaiswal's group further investigated roles of other Src kinase subfamily members (Src, Yes and Fgr) and the Lyn subfamily for controlling Nrf2 gene expression. They elucidated that all four Src subfamily kinases (Src, Yes, Fyn and Fgr) but not Lyn phosphorylate Nrf2 Tyr568 and lead to the nuclear export, ubiquitination and degradation of Nrf2 [20]. While there is controversy as to the direct interaction of GSK-3β with Nrf2, these data still indicate that when
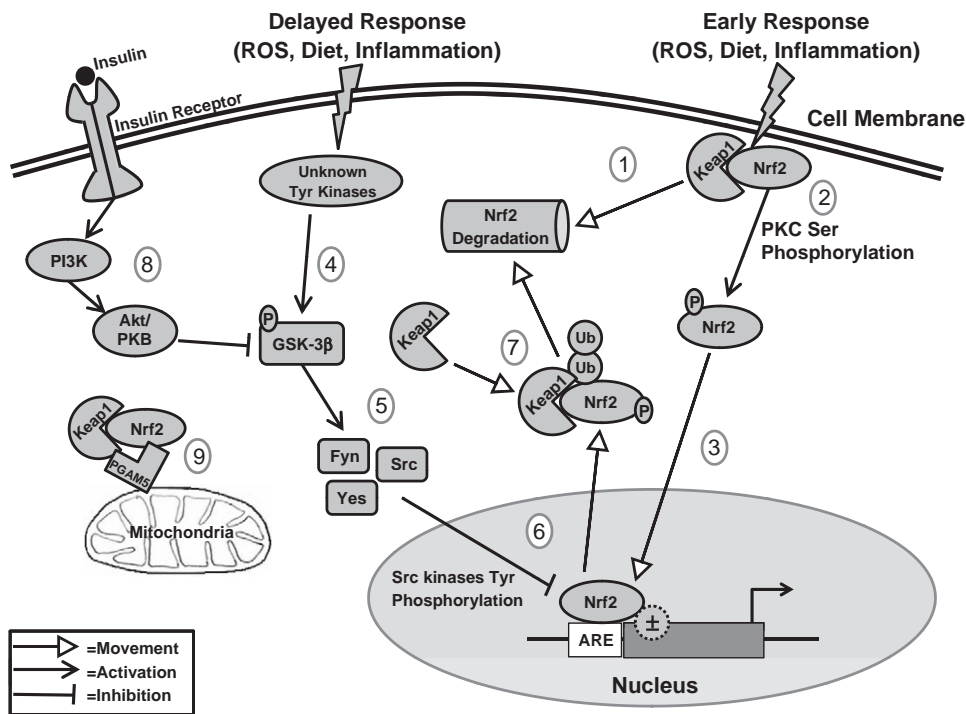

Fig. 1. Schematic diagram of Nrf2 regulation. This schematic diagram summarizes the signaling and proteins involved in the regulation of Nrf2, how this affects Nrf2 activity and its movement between the cytoplasm and nucleus. The open arrow represents movement, the pointer arrow represents activation, and the "T" arrow represents inhibition of proteins. (1) Typically, Nrf2 is sequestered to the cytoplasm through binding with Keap1 and continually shuttled to the proteasome for degradation. (2) Upon an early response to external stressors (0–4 h), Keap1 cysteine residues are oxidized or Nrf2 serine (Ser) 40 is phosphorylated by protein kinase C (PKC) [2,55]. (3) Nrf2 is then able to translocate into the nucleus and bind to ARE responsive genes in order to increase or decrease (±) their expression [2,5,13,16]. (4) Subsequently, a delayed response to external stressors causes the phosphorylation of GSK-3β by unknown tyrosine (Tyr) kinases. (5) GSK-3β then activates Src kinases, allowing for their translocation into the nucleus. (6) These Src kinases phosphorylate Nrf2 Tyr568 which allows for nuclear export, (7) ubiquitination and degradation of Nrf2 [20]. (8) However, if the insulin receptor signaling is initiated, GSK-3β activity is inhibited [17]. (9) Keap1 is also able to regulate Nrf2 activity through sequestration with PGAM5 to the mitochondria [8,9].

GSK-3β activity is blocked by insulin signaling, the Nrf2 signaling would then be up-regulated. Therefore, Nrf2 signaling may be coupled to insulin signaling and obesity-induced insulin resistance.

3. Regulation of adipocyte differentiation

There are conflicting data on whether Nrf2 enhances or inhibits adipogenesis and lipid metabolism. Several studies have utilized Nrf2 null mice and Keap1 knockout mice, and manipulated 3T3-L1 cells and Nrf2 activators in order to elucidate the function of Nrf2 in adipocyte cell biology. Pi and colleagues demonstrated that Nrf2 augments adipocyte differentiation [21]. Semiquantitative reverse transcriptase polymerase chain reaction and immunoblotting assays revealed an impairment in adipogenic genes (PPARγ, c/EBPα, CD36, lipoprotein lipase, aP2 and complement factor D) in primary Nrf2 knockout mouse embryonic fibroblasts (MEFs), Nrf2 knockdown 3T3-L1 cells and primary human adipocytes. However, knockdown of Keap1, thus preventing Nrf2 degradation, in the 3T3-L1 cells enhanced adipogenic gene expression [21]. Subsequent work by this laboratory shows that Nrf2 modifies the early events of adipogenesis by regulating the expression of *Cebpβ* [22]. In contrast, Shin and colleagues utilized immortalized MEFs from Nrf2 knockout and wild-type embryos and demonstrated an inhibitory role for Nrf2 in the adipogenesis process [23]. The Nrf2 knockout-immortalized MEFs had enhanced lipid accumulation and mRNA expression of terminal adipocyte differentiation effector genes, *Cebpα* and *Fabp4*, in comparison to wild-type and Keap1 knockout-immortalized MEFs [23].

Overall, the role of Nrf2 at the cellular level in adipogenesis is contradictory (inhibitory or stimulatory). The opposing works by Pi et al. and Shin et al. could be mainly due to the use of primary versus immortalized MEFs taken from two different Nrf2 knockout mouse models [21,23–25]. Generally, functional analyses are performed early in culturing at passage 3 for primary MEFs, while immortalized MEFs are utilized after passages 20–25 when the cells have accumulated enough genetic mutations that enable them to acquire the necessary growth advantages for cell survival and proliferation [26]. Therefore, in an Nrf2 knockout immortalized MEF cell line, it would be useful to reexpress Nrf2 by transfection to see if these Nrf2-rescued immortalized MEFs compared to wild-type immortalized MEFs. Moreover, it would be advantageous to make comparisons to differentiated adipose-derived stem cells and mature adipocytes isolated from adipose tissue from Nrf2 knockout and wild-type mice.

Additional evidences for Nrf2's role in adipogenesis and lipid metabolism are two high-fat (41% and 60% fat by calories for 12–14 weeks) feeding studies using Nrf2 knockout mice. The Nrf2 knockout mice in these two studies were protected from gaining as much weight as their wild-type counterparts, indicating that Nrf2 promotes adipogenesis [21,27]. Histological sections also revealed that the Nrf2 knockout adipocytes were smaller in size but higher in number than the wild-type adipocytes [21]. There has been one report, though, that demonstrated that Nrf2 knockout mice gained weight equally to wild-type mice. However, these mice were on a 35% high-fat diet for only 4 weeks [28]. This group also showed that the Nrf2 mRNA expression and the expression of two Nrf2 targets, NAD(P)H:quinone oxidoreductase 1 (NQO1) and Gstm6, were decreased in the wild-type mice on the high-fat diet [28].

Gaikwad and colleagues have shown in NQO1 knockout mice that the abdominal adipose tissue is significantly less in comparison to the wild-type mice. They hypothesize that the decrease in adipose tissue is due to an altered intracellular redox state supported by the higher ratios of NAD(P)H:NAD(P) found in the NQO1 knockout mice. Higher levels of NAD(P)H can presumably inhibit the pentose phosphate pathway which lowers the levels of pyridine nucleotides and therefore decreases gluconeogenesis and fatty acid metabolism [29].

Overall, high-fat feeding studies with Nrf2 knockout mice argue a stimulatory role for this transcription factor in adipogenesis. However, one must be cognizant that the ablation of Nrf2 from the whole mouse could be affecting multiple levels of the mouse's metabolic pathways and the result of reduced adipose depots or overall weight could be an adverse side effect [21,27,28]. One must also be aware that the amount of weight or fat gained may be directly correlated to the fat amount and composition (i.e., lard vs. milk fat) being used in the diet and the length of time the mice are on the diet. Therefore, if we want to more specifically address Nrf2's role in adipogenesis, it could be more useful to develop a Cre-lox mouse model system crossing Fabp4-cre mice [B6.Cg-Tg(Fabp4-cre)1Rev/J; The Jackson Laboratory] with loxP-Nrf2 targeted mice in order to specifically knockout Nrf2 from adipose tissues.

4. Regulation of liver energy metabolism

The liver is a major site of lipid and carbohydrate metabolism in addition to its detoxification functions. Thus, it is not surprising that, in addition to regulating hepatic antioxidant defenses, Nrf2 would also modify energy metabolism pathways in the liver. Through genetic and pharmacological manipulations that ablate or increase activity of the Nrf2 pathway, data indicate that Nrf2 activity suppresses the expression of multiple genes and subsequent proteins needed for lipid synthesis and that Nrf2 positively regulates cellular levels of the critical pyridine nucleotide cofactor NADPH.

That Nrf2 functions as a regulator of hepatic lipid metabolism was first demonstrated by Tanaka and colleagues [28]. This work tested the hypothesis that loss of Nrf2 exacerbates the hepatic effects of high-fat feeding such as increasing oxidative damage and steatotic markers (i.e., triglycerides and cholesterol). In these studies, Nrf2 knockout and wild-type mice were fed a high-fat (35% fat w/w from lard, approximately 65% of total calories from fat) or control (4% fat w/w, 7.5 % fat-based calories) diet for 1 week and for 4 weeks. After 4 weeks, serum cholesterol and free fatty acid levels were elevated in wild-type and Nrf2 knockout mice. However, serum cholesterol was lower in the high-fat-fed knockout mice in comparison to the high-fat-fed wild-type mice. Hepatic triglyceride content was elevated by the high-fat diet over the control mice, but there was no difference in the Nrf2 knockout animals on either the high-fat diet or the control diet. Hepatic free fatty acid was slightly elevated in the knockout mice on the high-fat diet, and additionally, hepatic malondialdehyde was approximately 25% greater in Nrf2 knockout over wild-type mice on the high-fat diet. Examination of hepatic gene expression revealed that the genes for multiple proteins associated with elevated lipid synthesis such as fatty acid synthase (FAS), fatty acid elongase and acetyl-CoA carboxylase 1, and sterol regulatory element binding protein-1c (SREBP-1c) were elevated by lack of Nrf2 under elevated fat. On the other hand, PPARγ was reduced in normal-fat- and high-fat-fed animals [28].

Wu and colleagues created a 'gene dose–response' model of Nrf2 by using the livers from Nrf2 knockout mice, wild-type mice, Keap1 knockdown (Keap1-KD) mice with enhanced Nrf2 activation and Keap1-hepatocyte knockout (Keap1-HKO) mice with maximum hepatic Nrf2 activation in order to examine how the level of Nrf2 altered the expression 52 lipid biosynthesis genes and 21 fatty acid synthesis genes [30]. Their results similarly posed Nrf2 in an inhibitory role for lipid and fatty acid synthesis. Of the lipid biosynthesis genes, 36 were expressed at higher levels in the Nrf2 knockout mice, but the expression levels were lower in the Keap1-HKO mice. Correspondingly, there were 14 fatty acid synthesis genes also highly expressed in the Nrf2 knockout mice in comparison to the Keap1-HKO mice, while 7 other lipid synthesis genes and 1 fatty acid synthesis gene were more highly expressed in the Keap1-HKO mice than the Nrf2 knockout mice [30].

Studies in which the Nrf2 pathway is chemically activated over basal levels complement those data examining knockout of Nrf2.

Studies by Shin and colleagues have used the potent Nrf2 inducer oleanic triterpenoid 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-IM) and examined hepatic gene expression and activity for lipid synthesis [27]. These studies demonstrated that the expression of genes for acetyl-CoA carboxylase (ACC 1 and ACC 2) and the activity for FAS were suppressed by CDDO-IM but only under high-fat-fed conditions. Yates and colleagues employed a similar approach comparing CDDO-IM to liver-specific suppression of Keap1, thus inducing Nrf2 activity. In this work, genes ACC1, fatty acid elongase, FAS and ATP citrate lyase were all suppressed when elevating Nrf2 by CDDO-IM and Keap1 inactivation [31].

Proteomic analyses comparing hepatic proteins from Nrf2 knockout and wild-type mice solidify a role of Nrf2 in regulating hepatic lipid metabolism and the cofactor acetyl-CoA. The expression of several lipid synthesis proteins such as FAS and acyl-CoA desaturase was elevated by lack of Nrf2. Interestingly, the content of the enzyme ATP-citrate lyase, a cytosolic acetyl-CoA synthetic enzyme, is elevated by lack of Nrf2. Thus, Nrf2 mice may have more acetyl-CoA present for lipid synthesis and perhaps for acetyltransferase activity. However, analyses of CoA intermediaries in Nrf2 knockout mice have not been performed.

The data above demonstrate that Nrf2 is a negative regulator of lipid synthesis. However, a potential mechanism underlying this negative regulation is suggested by the work of Huang and colleagues and Kay and colleagues [5,32]. Huang et al. showed that Nrf2 positively regulates the nuclear receptor small heterodimer partner (SHP) by demonstrating in 6-month-old Nrf2 knockout mice that the mRNA expression of SHP was decreased [32]. Additionally, an SHP luciferase assay demonstrated that coexpressing Nrf2 with coactivator Brahma-related gene 1, p300, Src1 or Src3, but not Nrf2 alone, allowed for enhanced activity of SHP. Lastly, they examined the hepatic mRNA expression of lipid metabolic genes PPARγ, FAS, Scd1 and SREBP-1c and suggested that their regulation was by Nrf2 since the mRNA expressions were all decreased in the 6-month-old Nrf2 knockout mice in comparison to wild-type mice [32]. Kay et al. also demonstrated a decrease in SHP mRNA expression in Nrf2 knockout mice (age not given) [5]. However, in contrast to the work of Huang et al., Kay et al. mechanistically demonstrated that there is specifically a negative regulation of the mRNA expression of lipogenic genes (FAS, SREBP-1c, acetyl-CoA carboxylase) when liver X receptor-α activity is inhibited by enhanced SHP activity [5]. The enhanced SHP activity is a result of Nrf2 activating the farnesoid X receptor (FXR) by deacetylation and disassociation from the p300 acetylase [5]. The p300 acetylase can block gene transcription through acetylating of various transcription factors. These data are very intriguing because this is the first demonstration of Nrf2 altering gene expression through direct association with another protein in addition to the commonly known binding of Nrf2 to the ARE [5].

While previous data demonstrate a direct stimulation of Nrf2 activity via the insulin signaling pathway in cardiomyocytes, a similar mechanism has not been directly shown in adipocytes and hepatocytes [33]. The relationship between Nrf2 and the insulin signaling pathway becomes important in the context of insulin resistance, which has been recently reviewed by Yu and colleagues [34]. A conundrum exists, however, in the roles that Nrf2 and insulin have in lipid synthesis. One would predict that Nrf2 would stimulate lipid synthesis since insulin signaling leads to the increase of lipogenesis and fat storage; nevertheless, the data above demonstrate that Nrf2 instead has an inhibitory role in lipid synthesis [35].

5. Role of Nrf2 in mitochondrial biogenesis

Recent experiments indicate that activation of the Nrf2 pathway enhances mitochondrial biogenesis. Owing to the central nature of mitochondria to whole body energy metabolism, means to increase the number of mitochondria and mitochondrial metabolism may be used to treat obesity. Mitochondrial biogenesis and mitochondrial protein expression are an interplay between nuclear and mitochondrial signaling and involve proteins such as AMP-kinase, peroxisome proliferator-activated receptor γ coactivator (PGC)-1α, PGC-1β, nuclear respiratory factor (NRF)-1 and NRF-2 (see recent reviews: [36–40]). NRF-1 and NRF-2 are critical transcription factors that induce expression of mitochondrial respiratory proteins [37,39,40].

Research by Piantadosi and colleagues shows that activation of Nrf2 allows for enhanced mitochondrial biogenesis in cardiomyocytes owing to multiple Nrf2 binding sites in the NRF-1 promoter [41]. In these studies, it was demonstrated that carbon monoxide (CO), a product of heme oxygenase 1 activity, elevates mitochondrial DNA copy number in an Nrf2-dependent manner. In this pathway, CO elevates mitochondrial H_2O_2 production that in turn activates the PKB/Akt kinase. Akt in turn phosphorylates (and thus inactivates) GSK-3β, allowing for Nrf2 to translocate to the nucleus [41]. Another study has found that Nrf2-dependent mitochondrial biogenesis occurs in the hippocampus in response to acetyl-L-carnitine treatment; however, the mechanisms connecting acetyl-L-carnitine to Nrf2 activation are unclear [42]. More data are needed to explore the role of Nrf2 activation to enhance mitochondrial number and activity, yet data (see below) indicate that Nrf2 activation enhances energy expenditure.

6. Can dietary factors alter metabolism through Nrf2 pathway activation?

The data presented above indicate that activation of Nrf2 is able to shift cellular metabolism such that mitochondrial DNA copy number is elevated, hepatic fatty acid synthesis is decreased and adipocyte hypertrophy is reduced. The activation of the Nrf2 pathway by phytochemicals such as the isothiocyanate sulforaphane is well documented and has been reviewed extensively with reference to cancer chemoprevention [4,43]. Growing attention is being paid towards activation of the Nrf2 pathway as a means of subsequently modifying cellular metabolism.

To this extent, studies have used the potent Nrf2 inducer CDDO-IM to determine if pharmacological activation of Nrf2 may reduce adiposity in high-fat-fed mice. Data by Shin and colleagues demonstrate that treatment of mice with CDDO-IM fed a high-fat diet (60% kcal of fat calories) prevents a large increase in body weight, an effect blunted in Nrf2 knockout mice [27]. Interestingly, CDDO-IM did not alter body weight of wild-type control-fat-fed (10% kcal) mice but did reduce the body weights of Nrf2 knockout mice on the normal-fat diet [27].

Similarly, oltipraz, another Nrf2 inducer, blunted high-fat-diet-induced increases in body fat deposition and insulin resistance [44]. These studies used C57Bl/6J mice fed a high-fat (45%-fat-derived calories) versus a 10% fat-calorie control. However, the source of fat was not reported [44].

The overall physiological effects of CDDO-IM appear to be the result of multiple mechanisms. CDDO-IM-treated mice on a high-fat diet have higher energy expenditure and a decrease in the expression of hepatic lipid synthesis genes than untreated mice on a high-fat diet [27]. On the other hand, there was no effect of CDDO-IM on lipid synthesis genes in white adipose, although the GSTa1 gene expression was elevated several fold, indicative of Nrf2 pathway activation. The fact that GSTa1 expression was elevated in white adipose without effects on lipid synthesis genes suggests that adipose effects of CDDO-IM in the whole animal context are secondary to changes in metabolism in the liver and likely other tissues such as muscle [27]. CDDO-IM also reduces the expression of the gene for the glycolytic enzyme pyruvate kinase [31]. Pyruvate kinase synthesizes pyruvate for the Kreb's cycle. A decrease in pyruvate could shift a cell to the use of fatty acids. However, these data

contrast with those showing a decrease in pyruvate kinase gene expression in Nrf2 knockout animals [45].

The pharmacological activation of the Nrf2 pathway opens the possibility that other dietary Nrf2 activators such as sulforaphane may also have effects on cellular lipid metabolism and total energy expenditure. As mentioned above, acetyl-L-carnitine induces mitochondrial biogenesis in an Nrf2-dependent manner [42]. Human genetic polymorphisms leading to higher activation of Nrf2 through disrupting the Nrf2-Keap1 interactions have also been identified [46,47]. The extents to which these polymorphisms lead to changes in lipid synthesis and energy expenditure are not known. On the other hand, these polymorphisms are also linked to increases in multiple cancers [46,47].

Metabolomic studies focusing on changes in the substrates and products of lipid and glucose metabolic pathways are needed. Importantly, the basis for the higher energy expenditure has not been elucidated. Since skeletal muscle and cardiac muscle are major sites of glucose and fatty acid utilization, subsequent studies need to address changes in muscular gene expression, mitochondrial physiology or mitochondrial biogenesis in these tissues.

7. Macronutrient control of Nrf2

Might the source of calories also impact Nrf2 activation? A study by Tanaka and colleagues demonstrated that a higher-fat diet (35% lard w/w) in mice reduces hepatic levels of mRNA for Nrf2 and NQO1 compared to control mice fed a normal-fat diet (4% w/w) [28]. Another study by Toye and colleagues similarly demonstrated that a high-fat diet (32% lard oil, 8% corn oil) reduced the hepatic mRNA levels for several GSTs and aldo–keto reductase 1C19 versus control mice receiving a "standard carbohydrate chow"[48]. Studies by Yu and colleagues demonstrated that a high-fat diet (45% fat calorie, fat source not reported) decreased nuclear Nrf2 content and increased Keap1 expression, leading to a decrement in Nrf2 activity as evidenced by a decrease in heme oxygenase 1 in the high-fat-fed animals [44]. Lastly, Kay and colleagues demonstrated a decrease in Nrf2, FXR and SHP mRNA expression when mice were placed on a high-fat diet (25% lard, 2.5% soybean oil) [5].

On the other hand, a study by Huang and colleagues demonstrated that a high-fat diet consisting of 42% of fat calories from milk fat doubled hepatic Nrf2 mRNA levels [32]. These latter results support data by Kim and colleagues demonstrating by microarray that a high-fat diet with 36% of calories derived from soybean oil increases the mRNA coding for Nrf2 and the glutathione synthetic enzyme glutamate cysteine ligase catalytic subunit, glutathione S-transferase (GST) M2 and GST M6 [49].

The mechanisms underlying these changes in transcript levels are unclear. Conceivably, a high-fat diet and insulin resistance can reduce Nrf2/ARE pathway activity. Translocation of Nrf2 to the nucleus can be enhanced through activation of the insulin/phospho-Akt pathway [50–52]. On the other hand, unlike lard, soybean oil contains appreciable amounts of n-3 fatty acids which in some cases can ameliorate insulin resistance and whose oxidation products can activate the Nrf2 pathway [50–52]. These oxidation products include electrophilic α,β-unsaturated aldehydes and cyclopentenones (α,β-unsaturated ketones) that activate Nrf2 in part through GSH depletion and/or direct modification of Keap1 [52–54]. These data are intriguing as they imply that the macronutrient energy source may regulate cellular antioxidant defense responses and that macronutrient composition may enhance or reduce the body's ability to resist disease states associated with elevated oxidative stress.

8. Issues

Several questions remain, however. How applicable are gene expression data to actual protein content and function? Is there a

difference between high-fat diets employing lard (mostly saturated and monounsaturated fatty acids) and those containing appreciable levels of n-3 fatty acids or n-6 fatty acids? The experiments above do have flaws found commonly in the obesity research literature. Diet composition and diet consumption are not adequately reported. Are the data above the result of changes in macronutrient intake or due to increases in caloric consumption? Without more detailed diet information, it is difficult to make firm conclusions.

9. Conclusions

Recent data clearly show that there is an interaction of the Nrf2-ARE pathway with energy metabolism such that activation of this pathway allows for elevated energy utilization. Conflicting data exist for the role of Nrf2 in adipose tissue and adipocyte cell biology, and there are scant data elucidating the function of the Nrf2 pathway in skeletal muscle. Evidence suggests that fatty acid content of the diet modulates Nrf2 activation, an area needing more study given the differing physiologic responses of saturated fatty acids and polyunsaturated fatty acids, particularly n-3s. Given the pressing biomedical issue of obesity, understanding the relationship of Nrf2 and energy metabolism in cells and tissues, as well as from a systems approach, will provide novel insights for nutritional interventions for obesity and diabetes.

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