Emerging Role of Nrf2 in Adipocytes and Adipose Biology\textsuperscript{1–3}

Kevin S. Schneider and Jefferson Y. Chan\textsuperscript{*}
Department of Laboratory Medicine and Pathology, University of California, Irvine, CA

**ABSTRACT**

Maintenance of a balanced redox state within the cell is of critical importance to a wide variety of biological systems. Nuclear factor erythroid-derived 2-like 2 (Nrf2) is a critical regulator of key aspects of the antioxidant defense pathway and has long been a subject of interest regarding conditions of chronic stress such as inflammation and cancer. Recent data have emerged demonstrating that oxidative stress and Nrf2 also play critical roles in the biology of adipose tissue. This review examines data identifying the roles of Nrf2 and oxidative stress in the biological process of adipocyte differentiation as well as the implications of Nrf2 modulation on obesity. Working to understand the complex interplay among Nrf2, oxidative stress, and adipose biology could lead to a variety of possible treatments for obesity and other related disorders. \textit{Adv. Nutr.} 4: 62–66, 2013.

**Introduction**

Obesity and related disorders, including diabetes and metabolic syndrome, are of growing interest and concern due to their increasing prevalence among the populations of the world. There is a drive to understand the complex interactions among mitochondrial function, metabolism, insulin signaling, and the ability of the body to maintain a balanced redox state in the face of various damaging by-products of normal cellular processes. It is becoming more apparent that regulation of the redox state of the cell and the removal of reactive oxygen species (ROS)\textsuperscript{4} play a pivotal role in the regulation of energy homeostasis within the body, with dramatic consequences for the organism as a whole.

Nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor originally discovered as one of the key antioxidant regulators within the body, has long been critical to this interest in the regulation of metabolic ROS. The primary function of Nrf2 is to respond to oxidative stress by activating antioxidant genes. Due to its involvement in cytoprotection within the body, Nrf2 has been widely studied for its role in cancer and inflammatory diseases. However, recent evidence has shown that the role of Nrf2 may stretch beyond that of oxidative stress protection. This review presents current data demonstrating the expanding roles of Nrf2 and oxidative stress in adipose tissue development and function.

**Current status of knowledge**

**Nrf2 and oxidative stress response**

Nrf2 is a member of the Cap-n-Collar subfamily of bZIP transcription factors that also includes p45NFE2, Nrf1, and Nrf3 (1,2). Under basal conditions, Nrf2 remains sequestered in the cytoplasm, bound to the Kelch-like ECH-associated protein 1 (Keap1). This association with Keap1 leads to the ubiquitination and subsequent proteasomal-mediated degradation of Nrf2. However, in response to oxidative stress, key cysteine residues of Keap1 are modified, resulting in the release of Nrf2 from Keap1. Upon release from Keap1, Nrf2 is free to translocate into the nucleus. In the nucleus, Nrf2 is able to form heterodimers with other transcription factors such as small Maf proteins (sMaf), JunD, and activation transcription factor 4 (ATF4) to bind and activate gene transcription via cis-acting antioxidant response elements present in the promoters of key cellular defense genes (3–6). These genes include many detoxification

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\textsuperscript{4} Abbreviations used: GCLM, regulatory subunit of \textit{g}-glutamate cysteine ligase; GPX-1, glutathione peroxidase 1; GSH, glutathione; NOX4, NADPH oxidase 4; Nrf2, nuclear factor erythroid 2-related factor 2; ROS, reactive oxygen species; SFN, sulforaphane.

\textsuperscript{*} To whom correspondence should be addressed. E-mail: jchan@uci.edu.

enzymes such as NAD(P)H quinone oxidoreductase 1 (NQO1) and heme oxygenase 1 (HO-1) as well as antioxidant enzymes such as the catalytic and regulatory subunits of γ-glutamate cysteine ligase (GCLM). Mice that lack the Nrf2 gene have no obvious outward defects. However, they have a tendency to develop autoimmune disorders and have increased susceptibility to toxicant-induced diseases, including heavy metal toxicity (7–10).

Oxidative stress and adipose tissue

Adipocyte differentiation and function have been shown to be affected by cellular redox status, which can be influenced by a variety of external and internal sources. For example, oxidative stress has been shown to directly modulate adipose cell differentiation. Treatment with low doses of hydrogen peroxide inhibited adipose differentiation of 3T3L1 cells. However, higher doses of hydrogen peroxide markedly induced differentiation (11). Further demonstrating the role of ROS, insulin-dependent adipocyte differentiation shows a dramatic increase in the amount of ROS within cells as well as increased NADPH oxidase 4 (Nox4), a key enzyme involved in production of cellular ROS (12). To further explore the role of Nox4 and ROS in adipocyte differentiation, Nox4 small interfering RNA knockdown in 3T3L1 cells was shown to be sufficient to prevent insulin-induced differentiation, whereas overexpression of Nox4 resulted in increased levels of adipocyte differentiation, mirroring previous treatments with hydrogen peroxide (12). Whether these discrepancies in differentiation are due to a direct result of ROS or from modulation of key stress response genes remains to be fully explored.

In addition to oxidant-induced oxidative stress, antioxidant genes have also been shown to play a role in adipogenesis. NQO1 is a key oxidative stress response gene under the control of Nrf2 and has been shown to prevent and detoxify dangerous quinones as well as help to reduce chromium (VI) toxicity (13–15). However, it appears that the role of NQO1 reaches beyond that of protection against dangerous metabolites and heavy metals. Recent work has demonstrated that adipose tissue mass is decreased in NQO1 knockout mice and this reduction in adipose tissue is hypothesized to be due to the role that NQO1 plays in maintaining the critical balance between NAD(P) and NAD(P)H (16). NQO1 is responsible for using NAD(P)H as an electron donor to catalyze the 2-electron reduction of damaging substances and quinones (16). The disruption in NAD(P) and NAD(P)H balance through the loss of NQO1 could potentially result in a decrease in gluconeogenesis and fatty acid metabolism, leading to altered lipid deposition (16). It is worth noting that although NQO1 −/− mice had decreased abdominal adipose tissue, demonstrating an obesity effect, they were, however, insulin resistant, indicating that the role of NQO1 in adipose tissue function is complex. The role of NQO1 has also been explored in adipocyte differentiation in vitro. NQO1 protein expression is induced during the initial stage of adipocyte differentiation and subsequently decreases after d 4 in culture. This expression pattern is required for the normal adipocyte differentiation of 3T3L1 cells (17). Treatment of 3T3L1 cells throughout the entire differentiation process with sulforaphane (SFN), an Nrf2 activator compound, resulted in an induction of Nrf2 and NQO1 and inhibited TG accumulation and adipocyte maturation. More specifically, it was found that inducing NQO1 at d 4, when amounts normally drop, elicited the same inhibitory effect, resulting in decreased hypertrophy of the adipocytes. These data demonstrate a complex and important temporal element in oxidative stress, NQO1, and normal adipocyte differentiation.

There is also an antiobesogenic effect in mice lacking glutathione peroxidase 1 (Gpx1). Gpx1 protects cells from oxidative stress by catalyzing the reduction of hydroperoxides. Gpx1 null mice had protection against diet-induced obesity and displayed enhanced insulin signaling (18). It is suggested that oxidation of phosphatase and tensin homolog by greater amounts of ROS led to increased insulin-stimulated glucose uptake in this mouse model. In support of ROS being the driving force behind the observed phenotype, it was shown that a diet supplemented with N-acetyl cysteine, a free radical scavenger, normalized ROS amounts in mutant tissues as well as caused insulin resistance in Gpx1 −/− mice.

It is accepted that aging is associated with increased levels of oxidative stress, which results in part from a decrease in the concentration of glutathione (GSH), a major source of reducing equivalents in mammalian cells. This decrease in GSH due to aging is associated with a wide variety of pathologies, including cancer and cardiovascular, inflammatory, metabolic, and neurodegenerative diseases (19). In addition, it has also been shown that aging increases levels of oxidative stress within adipose tissue, resulting in an inhibition of adipogenesis and subsequent decrease in adipose tissue mass (20). This age-related decline in GSH concentrations and the subsequent decrease in adipose tissue mass can be recapitulated in vitro by depleting cells of GSH using buthionine sulfoximine. Reduction of GSH and the subsequent increase in oxidative stress results in a severe inhibition of adipogenesis across many cell types, including 3T3L1 preadipocytes, as well as primary murine preadipocytes, with all cells showing a delayed onset of adipogenesis and a severe decrease in downstream targets of mature differentiated adipocytes such as aP2 (20).

Not surprisingly, mice lacking GCLM, the regulatory subunit of the glutamate-cysteine ligase enzyme involved in GSH synthesis, also display a resistance to high-fat diets (21). GCLM knockout mice had a lower weight when fed a high-fat diet, an increase in oxygen consumption, decreased hepatic lipid accumulation, and maintenance of insulin sensitivity. Taken together, these studies demonstrate examples of oxidative stress providing protection against obesity through a variety of targets and pathways. With Nrf2’s capacity to regulate so many of the above genes involved in oxidative stress, it serves as a very attractive target for understanding the role of oxidative stress in adipose tissue development and function.
Nrf2 and adipogenesis

Adipogenesis and adipocyte function is a relatively new realm of study in relation to Nrf2 and there have been a number of exciting, albeit conflicting, data indicating that Nrf2 may play a pivotal role in adipose biology, both directly and indirectly. A multitude of cell models have been used to examine the role of Nrf2 in adipocyte differentiation. In a study by Pi et al. (22), loss of Nrf2 led to impaired adipogenesis in primary mouse embryonic fibroblasts, 3T3L1 preadipocytes, and human s.c. preadipocytes. Conversely, adipocyte differentiation of 3T3-L1 cells was enhanced by activation of Nrf2 through knockdown of its negative regulator Keap1 (22). Deficiency of Nrf2 also resulted in a decrease in expression of key adipogenesis genes, including PPARγ, C/EBPα, and downstream targets of mature adipocyte differentiation, and this effect is exerted through direct regulation of PPARγ and Cebpβ by Nrf2 (22,23). However, conflicting results have been reported showing that the loss of Nrf2 is associated with increased differentiation capacity. In these experiments, Nrf2−/− immortalized mouse embryonic fibroblast cells had markedly accelerated adipogenic differentiation compared with wild-type cell lines (24). This phenotype could be reversed by ectopic expression of Nrf2, resulting in a more delayed differentiation. As a final comparison, Keap1 null cells, which constitutively express higher levels of Nrf2, also had delayed adipose differentiation (24). The basis for these discrepant results is not known, but it has been suggested that it could be due in part to the different cell models used in the studies, with dissimilarities arising when comparing primary cells and immortalized cell lines. Nonetheless, the information provided by these cell-based experiments underscores the possible role of Nrf2 in adipose tissue development.

In the past, Nrf2 target genes have been identified using a variety of experiments, including gene expression microarrays; however, recent work using chromatin immunoprecipitation-sequencing techniques has identified a wide number of novel Nrf2 target genes involved in the immune response and apoptosis and identified binding sites for Nrf2 in RXRα (25). RXRα is a key nuclear retinoid receptor that heterodimerizes with many targets, including PPARs, RARα, RARβ, RXRβ, and LXR, to mediate the transcriptional regulation of genes involved in a wide variety of cellular processes such as cell cycle, differentiation, and growth (25). The interaction of Nrf2 with RXRα is of interest to the field of adipose biology for the role that RXRα plays in binding with PPARγ to drive the process of adipogenesis (25). Strong binding between Nrf2 and RXRα was observed and it was demonstrated that after treatment with SFN, there was a marked increase in RXRα target gene expression. Knockdown of Nrf2 resulted in a delayed expression of RXRα, which also resulted in an inhibition of adipogenesis similar to that observed by Pi et al. (22). However, the activation of Nrf2 by SFN also had inhibitory effects on the capacity of 3T3L1 cells to differentiate and this effect depended on not only the length of treatment but also the stage of differentiation when treatment occurred. This inhibitory effects caused by Nrf2 activation was less pronounced when treatment occurred.

Table 1. Oxidative stress and Nrf2 in the regulation of fat cell development and obesity

<table>
<thead>
<tr>
<th>Observation</th>
<th>System</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Inhibition of adipogenesis</td>
<td>Low-dose hydrogen peroxide on 3T3L1 cells</td>
<td>11</td>
</tr>
<tr>
<td>Increased adipogenesis</td>
<td>High-dose hydrogen peroxide on 3T3L1 cells</td>
<td>11</td>
</tr>
<tr>
<td>Increased adipogenesis</td>
<td>Hydrogen peroxide treatment and Nox4 overexpression</td>
<td>12</td>
</tr>
<tr>
<td>Decreased obesity</td>
<td>NQO1 −/− mice</td>
<td>15</td>
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<tr>
<td>Inhibition of adipogenesis</td>
<td>Nrf2/NQO1 overexpression using SFN</td>
<td>16</td>
</tr>
<tr>
<td>Decreased obesity</td>
<td>GPX1 −/− mice</td>
<td>17</td>
</tr>
<tr>
<td>Decreased obesity/ inhibition of adipogenesis</td>
<td>Decreased GSH concentrations due to aging or pharmacological inhibition via buthionine sulfoximine</td>
<td>19</td>
</tr>
<tr>
<td>Decreased obesity</td>
<td>GCLM −/− mice</td>
<td>20</td>
</tr>
<tr>
<td>Decreased obesity/ inhibition of adipogenesis</td>
<td>Nrf2 −/− mice and primary cells</td>
<td>21,22</td>
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<tr>
<td>Increased adipogenesis</td>
<td>Nrf2 −/− immortalized cells</td>
<td>23</td>
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<tr>
<td>Decreased adipogenesis</td>
<td>Nrf2 ShRNA knockdown/Nrf2 induction via SFN</td>
<td>24</td>
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<tr>
<td>Decreased obesity</td>
<td>Nrf2 activation with CDDO-imidazolide and oltipraz</td>
<td>25,26</td>
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<tr>
<td>Decreased obesity</td>
<td>Nrf2 −/− mice</td>
<td>27,28</td>
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*CDDO, 1(2-cyano-3,12-dioxooleana-1,9(11)-diien-28-oyl; GCLM, regulatory subunit of γ-glutamyl cysteine ligase; GPX1, glutathione peroxidase 1; GSH, glutathione; Nox4, NADPH oxidase 4; SFN, sulforaphane; ShRNA, short hairpin RNA.*
3 d after differentiation, suggesting the inhibitory effects produced by Nrf2 are linked with the early stages of adipogenesis. Similar to NQO1, there appears to be a temporal nature to the role of Nrf2 in adipogenesis and alteration of Nrf2 levels during the early stages of adipogenesis can have dramatic consequences for normal cell differentiation. Although it is not possible to rule out effects that SFN has on other pathways unrelated to Nrf2, these cell-based experiments underscore a role for Nrf2 and adipogenesis (Fig. 1).

**Nrf2 knockout mice and obesity**

A number of studies have also shown a link between Nrf2 and adiposity in mice. For example, administration of potent Nrf2 activators, 1-2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl-imidazolide and oltipraz, in mice resulted in protection from high-fat diet-induced obesity, suggesting that activation of Nrf2 promotes a leaner phenotype (26,27). In contrast to these results, mice lacking Nrf2 have been found to demonstrate a lean phenotype. Mirroring the data shown using cell lines, Pi et al. (22) demonstrated that mice deficient in Nrf2 had a marked decrease in adipose tissue mass and adipocyte size and were protected from high-fat diet-induced obesity. These findings are also supported by 2 other studies demonstrating Nrf2 knockout mice were protected from high-fat diet-induced obesity and had a higher sensitivity to insulin and altered metabolic profile with lower circulating glucose, HDL, and leptin concentrations (28,29).

While pharmacological activation of Nrf2 appears to promote a leaner phenotype, it is interesting to note that the CDDO-imidazolide study also observed that untreated mice lacking Nrf2 weighed less than their wild-type counterparts when fed a high-fat diet, suggesting an obesogenic role for Nrf2. Table 1 summarizes the recent findings presented on the role of Nrf2 in adipogenesis and obesity. Currently, it is not known whether these differences can be accounted for in part by off-target effects of pharmacological manipulation of Nrf2. Equally possible, the lean phenotype in Nrf2 knockouts may be secondary to altered cellular redox states resulting from loss of antioxidant defense or indirect effects of Nrf2 deficiency on pathways regulating adipocyte differentiation and function.

**Conclusions**

Recent data show that there is a complex interaction between redox state and adipose tissue biology. Nrf2 serves as a powerful tool for understanding this interaction between ROS and adipose tissue, because it sits at the crossroads of multiple interconnected pathways regulating the ability of the cell to react to a variety of endogenous and exogenous sources of stress. It is likely that the role of Nrf2 and oxidative stress in adipose tissue development and function is complicated and depends on many factors. These factors can include the multitude of gene targets affected both directly and indirectly by Nrf2, the various sources of oxidative stress, the temporal aspects of Nrf2 expression in adipose differentiation, and even the age and genetic background of the mice used. It should also be noted that a large amount of work is being done looking into many of the metabolic aspects of Nrf2. There are several informative reviews presenting other aspects of the ever-increasing role of Nrf2 in a variety of systems, including insulin signaling and lipid metabolism as well as mitochondrial biogenesis and the role of diet composition in Nrf2 pathway activity (30,31). Future research will undoubtedly lead to a greater understanding of the critical role that Nrf2 and oxidative stress play in obesity and its related disorders.

**Acknowledgments**

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**Literature Cited**

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