Adipose Deficiency of Nrf2 in ob/ob Mice Results in Severe Metabolic Syndrome

Peng Xue,1 Yongyong Hou,1 Yanyan Chen,1,2 Bei Yang,1,3 Jingqi Fu,1 Hongzhi Zheng,1,2 Kathy Yarborough,1 Courtney G. Woods,1 Dianxin Liu,4 Masayuki Yamamoto,5 Qiang Zhang,1 Peng Xue,1 Yongyong Hou,1 Yanyan Chen,1,2 Bei Yang,1,3 Jingqi Fu,1 Hongzhi Zheng,1,2
Kathy Yarborough,1 Courtney G. Woods,1 Dianxin Liu,4 Masayuki Yamamoto,5 Qiang Zhang,1
Melvin E. Andersen,1 and Jingbo Pi1

Nuclear factor E2-related factor 2 (Nrf2) is a transcription factor that functions as a master regulator of the cellular adaptive response to oxidative stress. Our previous studies showed that Nrf2 plays a critical role in adipogenesis by regulating expression of CCAAT/enhancer-binding protein β and peroxisome proliferator-activated receptor γ. To determine the role of Nrf2 in the development of obesity and associated metabolic disorders, the incidence of metabolic syndrome was assessed in whole-body or adipocyte-specific Nrf2-knockout mice on a leptin-deficient ob/ob background, a model with an extremely positive energy balance. On the ob/ob background, ablation of Nrf2, globally or specifically in adipocytes, led to reduced white adipose tissue (WAT) mass, but resulted in an even more severe metabolic syndrome with aggravated insulin resistance, hyperglycemia, and hypertriglyceridemia. Compared with wild-type mice, WAT of ob/ob mice expressed substantially higher levels of many genes related to antioxidant response, inflammation, adipogenesis, lipogenesis, glucose uptake, and lipid transport. Absence of Nrf2 in WAT resulted in reduced expression of most of these factors at mRNA or protein levels. Our findings suggest a novel role for Nrf2 in regulating adipose development and function, by which Nrf2 controls the capacity of WAT expansion and insulin sensitivity and maintains glucose and lipid homeostasis.

White adipose tissue (WAT) is an active organ that can store and release energy, maintain lipid and glucose homeostasis, and secrete a variety of factors that influence appetite, insulin sensitivity, inflammation, and many other pathways of biological and clinical significance (1). Excess accumulation of WAT is a risk factor for insulin resistance and type 2 diabetes. Conversely, defects in adipogenesis or lipogenesis in WAT as in lipodystrophy, which impair the capacity of WAT to expand, also can result in insulin resistance (2,3).

Nuclear factor E2-related factor 2 (Nrf2, also known as Nfe2l2) is a master regulator of the cellular adaptive response to oxidative stress (4–6). In response to oxidative and electrophilic stress, Nrf2 heterodimerizes with small Maf proteins and other basic leucine zipper proteins and binds to antioxidant response elements (AREs) in the promoters of many antioxidant and detoxification genes (7), thereby increasing their transcription. Evidence supporting the pivotal roles of Nrf2 in protecting against oxidative stress comes, in part, from studies conducted in Nrf2-knockout (Nrf2−/−) mice that revealed that these animals exhibit a severe deficiency in the coordinated gene regulatory program for adaptive antioxidant response resulting in high susceptibility to oxidative stress-related disorders and chemical carcinogenesis (8). Thus, the Nrf2-mediated antioxidant response represents an important cellular defense mechanism that serves to maintain intracellular redox homeostasis and to limit oxidative damage (5,9). In addition to liver, intestine, lung, and kidney, where detoxification reactions routinely occur (10), Nrf2 is abundantly expressed or highly inducible in human and mouse adipocytes and in WAT (11,12). However, other than detoxification and antioxidant defense, the exact function of Nrf2 in adipose tissues is not well-understood.

Our previous study (11) showed that mice deficient in Nrf2 possess decreased fat mass and are resistant to high-fat diet–induced obesity. In addition, we found that Nrf2 serves as an important transcriptional regulator of CCAAT/enhancer-binding protein β (C/EBPβ) (12) and peroxisome proliferator-activated receptor γ (PPARγ) (11) during adipocyte differentiation, suggesting that Nrf2 is one of the key transcription factors that controls adipogenesis. Defects in adipogenesis (e.g., caused by ablation of Ppary or suppression of C/EBP) are critical pathogenic factors of lipodystrophy, a syndrome characterized by total or partial fat loss associated with severe lipid and glucose abnormalities leading to diabetes with early cardiovascular, renal, and hepatic complications (13–15). To examine the role of Nrf2 in adipose function and metabolic syndrome, in the current study we determined the effect of ablation of Nrf2 on the development of obesity and associated metabolic disorders in leptin-deficient (ob/ob) mice, a model with an extremely positive energy balance. Interestingly, ob/ob mice with whole-body or adipocyte-specific ablation of Nrf2 displayed reduced WAT mass but had an even more severe metabolic syndrome characterized by hyperlipidemia, aggravated insulin resistance, and hyperglycemia. These findings provide further support that Nrf2 is a key transcription factor that controls WAT development and function, and thus affects insulin sensitivity, glucose tolerance, and lipid homeostasis. In light of the new function of Nrf2 in adipogenesis and its canonical role in adaptive antioxidant response, our results suggest a novel mechanistic linkage between metabolic syndrome and oxidative stress, opening the possibility that manipulation

From the 1Institute for Chemical Safety Sciences, The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina; the 2School of First Clinical Sciences, China Medical University, Shenyang, China; the 3College of Basic Medical Sciences, China Medical University, Shenyang, China; the 4Metabolic Signaling and Disease Program, Sanford-Burnham Medical Research Institute, Orlando, Florida; and the 5Department of Medical Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Japan. Corresponding author: Jingbo Pi, jpi@thehammer.org.
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of Nrf2 may prevent or treat obesity and associated metabolic syndrome.

**RESEARCH DESIGN AND METHODS**

**Mice.** Global Nrf2\textsuperscript{+/−} mice were developed as described previously (10) and back-crossed onto the C57BL/6J background for seven generations using alternating male and female stock mice from The Jackson Laboratories (JAX Stock No. 000664). The resulting Nrf2\textsuperscript{−/−} females were crossed with heterozygous male B6.129P2-\textit{Nkx2-5}\textsuperscript{+/-}ob/ob (ob/ob, JAX Stock No. 00632) to generate Nrf2\textsuperscript{−/−}:ob/ob and Nrf2\textsuperscript{−/+}/ob mice for the current study. To delete Nrf2 only in adipocytes, a line of C57BL/ 6J mice in which exon 5 of the Nrf2 gene was flanked by LoxP sites (Nrf2\textsuperscript{lox}\textsuperscript{5/Lox}) was generated as detailed in Supplementary Fig. 1A. The Nrf2\textsuperscript{lox}/lox\textsuperscript{5/Lox} mice were crossed with mice expressing Cre recombinase under the control of the adipocyte-specific Fabp4/4p2 gene promoter (B6.Cg-Tg(Fabp4-cre)1Rei/J; JAX Stock No. 005060). The resulting adipocyte-specific Nrf2\textsuperscript{−/−} mice [Nrf2\textsuperscript{(−/−)}], with the genotype of Nrf2\textsuperscript{lox}/lox\textsuperscript{5/Lox}\textsuperscript{Cre/Cre} or Cre\textsuperscript{−/−}, lacked Nrf2 expression in WAT and brown adipose tissues, but not in liver, skeletal muscle, pancreas, or lung (Supplementary Fig. 1B). Subsequently, the Nrf2\textsuperscript{(−/−)} mice were crossed with ob/ob mice to generate Nrf2\textsuperscript{(−/−)}/ob:ob and Nrf2\textsuperscript{(−/−)}/ob:ob mice. The mice were housed in virus-free facilities on a 12-h light/12-h dark cycle and were fed NIH07 chow-diet (Zeigier Brothers, Gardners, PA) and provided reverse-osmosis water ad libitum. Body weight and food consumption were determined weekly to allow calculation of cumulative food consumption. Genotyping was performed by PCR (primer sequences in Supplementary Table 1) using genomic DNA that was isolated from tail snips. All protocols for animal use were approved by the Institutional Animal Care and Use Committee of The Hamner Institutes and were in accordance with the National Institutes of Health guidelines.

**Measurement of blood glucose and plasma insulin.** After a 16-h period, fasting blood samples collected from tail bleeds were immediately analyzed for glucose using the FreeStyle Blood Glucose Monitoring System (TheraSense, Alameda, CA). Animals for plasma isolation and tissue collection were anesthetized by 2,2,2-trifluoroethanol (TFA; Sigma-Aldrich, St. Louis, MO) and Santa Cruz (Santa Cruz, CA), respectively. The molecular weight of each protein shown on the immunoblot was estimated based on the MagicMark XP Western Protein Standard (ThermoFisher Scientific) using the MagicMark XP Western Protein Standard (Invitrogen) on 12% Tris-Glycine gels. Antibodies for 

**RESULTS**

Global ablation of Nrf2 in ob/ob mice reduces body mass but aggravates insulin resistance and hyperglycemia. The BW of Nrf2\textsuperscript{−/−}:ob/ob, WT ob/ob (Nrf2\textsuperscript{+/-}:ob/ob), and their non-ob/ob littermates (Nrf2\textsuperscript{−/−}:WT or Nrf2\textsuperscript{+/-}:WT) was monitored during an 11-week period in mice 4–15 weeks of age. On a normal chow diet, Nrf2\textsuperscript{−/−}:ob/ob mice gained weight at a lower rate than Nrf2\textsuperscript{+/-}:ob/ob mice, such that they weighed substantially less from week 5 through week 11. Maximal differences were observed during week 8, at which time the body mass of the Nrf2\textsuperscript{−/−}:ob/ob mice was less than that of Nrf2\textsuperscript{+/-}:ob/ob mice by 23% in males (Fig. 1A) and by 26% in females (Supplementary Fig. 2A), respectively. Cumulative food consumption by Nrf2\textsuperscript{−/−}:ob/ob mice showed a similar rate as in Nrf2\textsuperscript{+/-}:ob/ob mice up to week 10 (Fig. 1B), but gradually decreased beyond this time point in females (Supplementary Fig. 2B). Compared with Nrf2\textsuperscript{+/-}:ob/ob mice, both male and female Nrf2\textsuperscript{−/−}:ob/ob mice had development of hyperglycemia and trended toward an increase in fasting plasma insulin and homeostatic model assessment for insulin resistance (Fig. 1C and Supplementary Fig. 2C–E). In addition, Nrf2\textsuperscript{−/−}:ob/ob mice exhibited insulin resistance demonstrated by a severely blunted response to intraperitoneal glucose tolerance test (Fig. 1F and Supplementary Fig. 2F) and intraperitoneal insulin tolerance test (Fig. 1G and Supplementary Fig. 2G).
Of note, four of 34 Nrf2+/−:ob/ob mice died between 8 and 12 weeks because of severe metabolic disorders exhibiting extraordinary hyperglycemia (499 mg/dL), dark yellow urine, and very low BW. Consistent with previous reports (11,19), Nrf2+/−:WT mice fed normal chow diet displayed slightly reduced BW gain, normal fasting blood glucose level, and insulin sensitivity compared with Nrf2+/+:WT mice (Fig. 1A and Supplementary Fig. 2).

Global ablation of Nrf2 in ob/ob mice reduces WAT mass and alleviates hepatic steatosis, but results in hypertriglyceridemia. Although Nrf2+/−:ob/ob mice still had development of adipose (Fig. 2A) and their visceral WAT pads, including retroperitoneal and epididymal (male) or gonadal (female) depots, were significantly smaller than those of Nrf2+/+:ob/ob mice (Fig. 2B and Supplementary Fig. 3A). Histomorphometric analysis of WAT displayed that Nrf2+/−:ob/ob mice had fewer small adipocytes in subcutaneous and epididymal WAT than Nrf2+/+:ob/ob mice (Fig. 2C and Supplementary Fig. 4), suggesting that deficiency of Nrf2 impairs the capability of adipocyte recruitment (20). Interestingly, Nrf2+/−:ob/ob mice were protected against hepatic steatosis, showing notably less accumulation of hepatic lipid droplets as well as smaller lipid droplet size than Nrf2+/+:ob/ob mice (Fig. 2C). In addition, Nrf2+/−:ob/ob mice displayed a substantially reduced intrahepatic triglyceride content (Fig. 2D and Supplementary Fig. 3B). In contrast to the reduced lipid deposition in liver, Nrf2+/−:ob/ob mice exhibited a trend toward hypertriglyceridemia, with a nearly 52.6% (males) and 39.6% (females) increase of fasting plasma triglyceride levels compared with Nrf2+/+:ob/ob mice (Fig. 2E and Supplementary Fig. 3C). When the data in males and females were pooled, a significant difference of plasma triglycerides levels between Nrf2+/+:ob/ob and Nrf2+/−:ob/ob mice was reached (not shown). Triglycerides in the skeletal muscle (Fig. 2F, Supplementary Fig. 3D) and plasma levels of free glycerol and free fatty acids (Supplementary Fig. 5) showed no significant difference between Nrf2+/+:ob/ob and Nrf2+/−:ob/ob mice.

**FIG. 1.** Ob/ob male mice with global Nrf2 deletion exhibit reduced BW, aggravated insulin resistance, and hyperglycemia. A: BW analysis of mice maintained on a chow diet. n = 8–11. *P < 0.05 vs. non-ob/ob mice with the same age. B: Cumulative food consumption. n = 3–8. C: Fasting blood glucose. n = 16–29. *P < 0.05 vs. non-ob/ob mice with the same genotype; †P < 0.05 vs. Nrf2+/−:ob/ob mice. D: Fasting plasma insulin. n = 3–5. E: Homeostatic model assessment for insulin resistance (HOMA-IR). n = 3–5. F: Intraperitoneal glucose tolerance test. Mice were challenged with 0.5 mg of glucose per gram of BW. n = 7–9. *P < 0.05 vs. Nrf2+/−:ob/ob mice with the same treatment. G: Intraperitoneal insulin tolerance test. Mice were challenged with insulin at 0.75 and 4 U/kg of BW in non-ob/ob and ob/ob mice, respectively. n = 8–15.
**Ob/ob mice with adipocyte-specific Nrf2 knockout exhibit a similar phenotype as Nrf2<sup>−/−</sup>:ob/ob mice showing reduced WAT mass, increased plasma triglycerides, insulin resistance, and hyperglycemia.** A reduction in fat, commonly found in genetically engineered mouse models, could be attributable to a number of biochemical abnormalities in other tissues. To substantiate a regulatory role of Nrf2 in adipose function and glucose homeostasis, a line of ob/ob mice with adipocyte-specific Nrf2 deletion was developed (Supplementary Fig. 1). The adipocyte-specific knockout of Nrf2 in ob/ob mice resulted in significantly diminished expression of Nrf2 in WAT (Fig. 3A). In agreement with the findings in Nrf2<sup>−/−</sup>:ob/ob mice, Nrf2<sup>(f)</sup><sup>−/−</sup>:ob/ob mice fed a chow diet showed a trend toward decreased BW (Fig. 3B) and significantly reduced WAT mass (Fig. 3C). Histomorphometric analysis of WAT demonstrated a trend in which Nrf2<sup>−/−</sup>(f):ob/ob mice had fewer small adipocytes in epididymal WAT than Nrf2<sup>−/−</sup>(f):ob/ob mice (Supplementary Fig. 6). Consistent with the phenotype of Nrf2<sup>−/−</sup>:ob/ob mice, Nrf2<sup>(f)</sup><sup>−/−</sup>:ob/ob mice exhibited significantly increased plasma triglycerides (Fig. 3F), reduced insulin sensitivity (Fig. 3G, H), and elevated fasting blood glucose levels (Fig. 3I). Thus, the severe metabolic syndrome of Nrf2<sup>−/−</sup>:ob/ob mice remains in Nrf2<sup>(f)</sup><sup>−/−</sup>:ob/ob mice, and the phenotype of Nrf2 deletion in ob/ob mice can be attributed, at least in part, to the lack of Nrf2 in adipose tissues. In contrast to the reduced lipid content in the liver of Nrf2<sup>−/−</sup>:ob/ob mice (Fig. 2C, D), adipocyte-specific ablation of Nrf2 did not significantly affect lipid deposition in the liver or skeletal muscle (Fig. 3D, E).

**FIG. 2.** Nrf2<sup>−/−</sup>:ob/ob male mice show reduced WAT mass and mild hepatic steatosis but trended increased plasma triglycerides. A: Representative images of fat tissues. Animal age is 10 weeks. B: Weight of WAT. Retroperitoneal and epididymal depots were measured. n = 11–18. Animal age is 8–15 weeks. C: Representative histological images of WAT and liver with hematoxylin and eosin (H&E) staining (20×). S-WAT, subcutaneous WAT; E-WAT, epididymal WAT. The white round areas on liver slides are lipid droplets. D–F: Levels of triglycerides in liver (D), plasma (E), and skeletal muscle (F), n = 6–7. Values in B, D, E, and F are mean ± SD. *P < 0.05 vs. non-ob/ob mice with the same Nrf2 genotype; #P < 0.05 vs. Nrf2<sup>−/−</sup>:ob/ob mice. (A high-quality color representation of this figure is available in the online issue.)
Ob/ob mice show enhanced adaptive antioxidant and inflammatory responses that are diminished by Nrf2 deletion. GSH is the most important and abundant redox buffer in cells (21). By scavenging peroxides, mainly through glutathione peroxidase-catalyzed reactions, GSH oxidation forms GSSG. Key enzymes involved in the de novo synthesis and regeneration of GSH include glutamate cysteine ligase catalytic and regulatory subunit, GSH synthetase, and GSH reductase which are all regulated, at least in part, by Nrf2 through the ARE (22–25).

Nrf2+/+::ob/ob mice expressed higher levels of Gclm and heme oxygenase 1 (Ho1) in WAT and Gclc, Ho1, and NAD(P)H:quinone oxidoreductase 1 (Nqo1) in liver than those in their non-ob/ob littermates (Fig. 4A; Supplementary Fig. 7). The absence of Nrf2 in mice either on C57BL/6J or on ob/ob background showed a trend toward reduced expression of these ARE genes. In addition, the expression of other antioxidant genes, such as superoxide dismutase 3 (Sod3), Gpx2, Gpx4, and thioredoxin reductase 1 (Txnrd1) displayed similar trends in WAT or liver (Supplementary Figs. 7 and 8), indicating ob/ob mice have enhanced adaptive antioxidant response that was diminished by Nrf2 deletion. Compared with Nrf2+/+::WT mice, Nrf2+/+::ob/ob mice showed a trend toward increased whole-blood and plasma GSH and GSSG levels (Fig. 4B and Supplementary Fig. 9), which were reduced by ablation of Nrf2. These changes were greater for plasma than whole blood.

Inflammation is associated with many pathologies, including obesity and insulin resistance (26). Nrf2+/+::ob/ob WAT showed a trend toward increased expression of several inflammatory response genes (Fig. 4A), including tumor necrosis factor α (Tnfa), interleukin 1β (I1β), and...
nitric oxide synthetase 2 (Nos2) compared with Nrf2+/+: WT mice, whereas the absence of Nrf2 markedly diminished their induction. In addition, the mRNA expression of many antioxidant and inflammatory response genes in WAT trended lower in Nrf2(−/−): ob/ob mice compared with Nrf2(+/+): ob/ob mice (Supplementary Fig. 10). Nrf2−/−:ob/ob mice show insulin resistance in WAT, which displays impaired adipogenesis. To determine whether ablation of Nrf2 in ob/ob mice results in insulin resistance in WAT, the key effector of insulin signaling cascade, phosphorylated AKT (p-AKT), was determined in epididymal WAT of mice treated with insulin. Insulin-stimulated p-AKT(S473) and p-AKT(T308) in WAT of Nrf2−/−:ob/ob mice (Fig. 5A, B) were significantly lower than those in Nrf2+/:ob/ob mice, indicating reduced insulin sensitivity in WAT of Nrf2−/−:ob/ob mice. To explore the mechanisms behind the insulin resistance in the WAT of Nrf2−/−:ob/ob mice, mRNA expression profiling in epididymal WAT of Nrf2−/−:ob/ob mice was determined by RT-qPCR. Because the metabolic phenotype of Nrf2−/−:ob/ob mice was consistent for both males and females (Figs. 1 and 2 and Supplementary Figs. 2–6), mRNA expression profiling only was performed in one gender, the male mice. In addition to the induction of antioxidant and inflammatory response genes that was observed in Nrf2+/+:ob/ob mice (Fig. 4A and Supplementary Fig. 8), the WAT of
Nrf2+/+ob/ob mice trended higher levels of many genes related to adipogenesis (Supplementary Fig. 11) and lipogenesis (Supplementary Fig. 12), including Pparγ2 and fatty acid synthetase (Fas), than levels in Nrf2+/+ mice. The absence of Nrf2 in ob/ob mice resulted in a trend toward reduced expression of most of these genes. In agreement with the findings in Nrf2+/+ob/ob mice, Nrf2(f)+/+ob/ob WAT also trended reduced levels of adipogenic and antioxidant response genes, including Pparγ2, adipose differentiation-related protein (Adfp), and Fas (Supplementary Fig. 13). In keeping with the reduced mRNA expression of adipogenic factors, the protein expression of PPARγ and GLUT4 in WAT of ob/ob mice also was significantly reduced by Nrf2 deficiency (Fig. 5C, D).

**DISCUSSION**

WAT is pivotal to lipid homeostasis and energy balance and regulates the flux of fatty acids to peripheral tissues by storing and hydrolyzing triglyceride under hormonal control (1,27). Here, we show that on the ob/ob background, ablation of Nrf2, globally or specifically in adipocytes, results in reduced WAT mass but leads to an even more severe metabolic syndrome showing insulin resistance, hyperglycemia, and hyperlipidemia. It appears that the extremely positive energy balance resulting from leptin deficiency uncovers a deleterious effect of Nrf2 deletion in adipose tissues.

Adipogenesis is a complex process in which multipotent mesenchymal stem cells first become committed to fibroblast-like preadipocytes and subsequently convert to mature spherical adipocytes with lipid accumulation (28–31). Terminal adipogenesis involves a sequential cascade of gene expression events coordinated by transcription factors that simultaneously induce tissue-specific gene expression and repress alternate cell fates (29,30). At the center of this network is the adipogenic factor PPARγ, which orchestrates the entire terminal differentiation process (28–30). C/EBPs, including C/EBPa, C/EBPβ, and C/EBPδ, belong to basic leucine zipper transcription factors and are expressed in adipocytes (29). C/EBPβ is transiently expressed and function at an early stage of differentiation by sensing adipogenic stimuli and initiating the expression of PPARγ and C/EBPa (32). C/EBPa and PPARγ form a positive feedback loop and act at a later stage by inducing and maintaining expression of adipocyte-specific genes, including Adfp, Fas, Cd36, and Glut4 (33). In the current study, many adipogenic genes exhibited a substantial increase in the WAT of ob/ob mice compared with non-ob/ob littermates. It appears that...
adipogenesis contributes to the hypertrophy of WAT and weight gain of ob/ob mice. The results that Nrf2−/−:ob/ob mice had fewer small adipocytes in WAT than Nrf2+/+;ob/ob mice and that whole-body or adipocyte-specific ablation of Nrf2 substantially reduced the expression of the adipogenic genes indicate that impaired adipogenesis occurred in the WAT of Nrf2−/−:ob/ob mice. This finding is consistent with our previous studies showing that Nrf2 plays a critical role in adipogenesis by regulating the expression of C/EBPβ and PPARγ (11,12).

Extreme defects in adipogenesis (e.g., attributable to complete ablation of Pparγ) lead to lipodystrophy (34). However, less extreme reduction of adipogenesis alone does not cause lipodystrophy. For example, a moderate reduction in adipogenesis caused by PPARγ/RXR antagonists, heterozygous deficiency of Pparγ, or other genetic variants in Pparγ reduce adipose tissue mass without causing lipodystrophy (35–39). Although Nrf2 plays important roles in regulating the expression of Cebpβ and Pparγ, the absence of Nrf2 does not completely abolish PPARγ activity and adipogenesis (11,12). Thus, knocking-out of Nrf2 reduces adipogenesis and also attenuates adipocyte hypertrophy and weight gain without causing an extreme reduction in adipose tissue mass (11,19). However, when adipose tissue expandability is limited by Nrf2 deficiency on a hyperphagic ob/ob background, the excess energy intake cannot be stored adequately in the adipose tissues and, consequently, leads to ectopic lipid accumulation in circulation. This phenotype, observed in Nrf2−/−:ob/ob and Nrf2(f)−/−:ob/ob mice, is consistent with the manifestations of P465L PPARγ mutant or Pparγ2-knockout mice on an ob/ob background (20,40).

It also suggests a synergistic interaction between Nrf2 deletion and a severe positive energy balance caused by the lack of leptin in inducing severe metabolic syndrome. Considering the critical roles of adipogenesis in adipose formation and in keeping healthy adipose function, impairment of adipogenesis resulting from a deficiency in Nrf2 could contribute to a more severe metabolic syndrome in Nrf2−−:ob/ob and Nrf2(f)−/−:ob/ob mice.

Oxidative stress, which is associated with many pathologies, including obesity and metabolic syndrome, can stem from a variety of sources, including overfeeding, high-fat diet, and exposure to certain environmental agents (41). Although potentially cytotoxic, reactive oxygen species (ROS) are important intracellular signaling molecules for cellular responses to a variety of physiological stimuli, including glucose sensing in pancreatic β cells (42) and insulin signal transduction in insulin-responsive cells (43). In adipose tissues, ROS promote the conversion from preadipocytes to mature adipocytes (12,44) and facilitate insulin action (43,45). These ROS-mediated biological signaling pathways could be adversely affected by enhanced Nrf2-ARE activity because ROS signaling intermediates should inversely correlate with the ROS-scavenging activity and antioxidant status in cells. When cells are chronically exposed to oxidative stressors, cellular ROS-scavenging capacity is adaptively upregulated, primarily through the activation of Nrf2 and subsequent transcriptional induction of a suite of antioxidant enzymes. The induced antioxidant enzymes, meant to maintain intracellular redox homeostasis and limit oxidative damage, may have the undesired effect of impeding the physiological role of ROS as signaling molecules. Thus, ROS, antioxidants, and the cellular adaptive antioxidant response seem to play counteracting roles in regulating adipose function.

Although antioxidants protect adipocytes from oxidative damage, they also may blunt aspects of ROS signaling, resulting in reduced adipogenesis and insulin resistance. In support of this idea, the absence of Nrf2 in non-ob/ob mice resulted in a slightly leaner phenotype with increased insulin sensitivity (11,19). This lean phenotype might be associated with lowered antioxidant expression and improved ROS signaling. Ob/ob mice have elevated oxidative stress demonstrated by the increased levels of blood GSH and GSSG and induction of many Nrf2-target genes in WAT and the liver. Thus, Nrf2-mediated adaptive induction of antioxidant enzymes in ob/ob mice might account for their moderately reduced insulin sensitivity and glucose intolerance. The activation of Nrf2 in WAT of Nrf2−−:ob/ob mice also suggests that oxidative stress-triggered Nrf2 activation may be required for adipose accumulation. Because Nrf2−−:ob/ob mice show lowered antioxidant expression in WAT and the liver, we expected that the mice have enhanced ROS signaling and improved insulin sensitivity. However, an impaired insulin sensitivity and even more severe metabolic disorders were observed in both Nrf2−−:ob/ob and Nrf2(f)−/−:ob/ob mice. This phenotype suggests that under challenge with extremely positive energy balance, the reduced capacity of WAT expansion is the dominant mechanism for their phenotype that cannot be fully compensated by enhanced ROS signaling from the Nrf2 deficiency.

Inflammation is involved in many aspects of the pathologies of obesity and metabolic syndrome (26). Nrf2-ARE signaling is involved in attenuating inflammation-associated pathogenesis (46). However, Nrf2 also is directly involved in the transcriptional regulation of proinflammatory cytokine Il6 (47). Nrf2 deficiency attenuates high-fat diet-induced inflammation in the stromal vascular and adipocyte fractions of adipose tissues (43). Thus, there appears to be potential cross-talk between Nrf2-mediated antioxidant response and inflammatory response. In the current study, although inflammatory response was augmented in WAT of ob/ob mice and may be involved in their moderate insulin resistance, substantially reduced expression of many inflammation-related genes in WAT of Nrf2−−:ob/ob and Nrf2(f)−/−:ob/ob mice indicated that their severe metabolic disorder is not attributable to inflammatory response.

In summary, our studies provide new insights into the mechanisms by which Nrf2 regulates adipose development and function. We found that Nrf2 controls WAT expandability and serves to maintain glucose and lipid homeostasis. Thus, this transcription factor, normally considered a regulatory protein for oxidative stress response, clearly has diverse roles, including control of adipogenesis. In addition, ROS, whose cellular concentrations decline with increases in Nrf2-regulated antioxidant gene expression, also affect insulin signaling. From our perspective, it has become increasingly clear that a full understanding of adipocyte function and obesity-associated metabolic disorders will require further investigations of the multiple interacting roles of Nrf2 in these physiological processes. In light of the involvement of Nrf2 in regulating adaptive antioxidant response and the association of metabolic syndrome with oxidative stress, we counsel caution in pursuing strategies to prevent or treat obesity and associated metabolic syndrome by manipulating Nrf2 function.
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P.X., Y.H., D.L., and J.P. designed the research. P.X., Y.H., Y.C., B.Y., J.F., H.Z., K.Y., and C.G.W. performed the experiments. P.X., Y.H., D.L., Q.Z., and J.P. analyzed the data. C.G.W., M.Y., Q.Z., M.E.A., and J.P. wrote the manuscript. J.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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