Chapter 8

Redox Modulation of Adipogenesis

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Abstract

Obesity and obesity-associated disorders have become globally prevalent. Knowing how adipose tissue forms is important for the intervention of obesity. In the last decades, numerous evidence have shown that redox status changes under the condition of obesity and in the process of adipogenesis. Alterations of both oxidant and antioxidant levels may influence the transformation from stem cell or preadipocytes to mature adipocytes. Redox system exerts "tridimensional" mechanism in the regulation of adipocyte differentiation, including transcriptional, epigenetic, and posttranslational modulations. However, the roles of redox system in the regulation of adipocyte differentiation are paradoxical. Therefore, we propose that restoration and maintenance of redox balance rather than simple prooxidant or antioxidant interventions are critical for the prevention and therapy of obesity and obesity-associated disorders.

Keywords: redox, adipogenesis, obesity, ROS, antioxidant

1. Introduction

To date, it is estimated that more than a billion adults are overweight and over 300 million adults are classified as obese worldwide [1]. Owing to the dramatic prevalence of obesity, there is a drastic increase in obesity-associated diseases, including hypertension, type 2 diabetes, dyslipidemia, kidney disease, heart disease, cancer, obstructive sleep apnea, and osteoarthritis. The adipose tissue, also called fat, includes white adipose tissue (WAT) and brown adipose tissue. Obesity is mainly caused by an excessive accumulation of fat mass in WAT to the extent that health may be adversely affected. WAT functions as both a metabolic and an endocrine organ that lies at the heart of a complex network participating in the regulation of various biological responses, including inflammation, immunity, endocrine, and metabolism [2].

Adipose tissue is a dynamic tissue, and the mass of adipose is generally determined by a balance of adipocyte proliferation, differentiation, enlargement, and lipolysis. Increased adipose

mass may result from the enhancement of adipocyte formation (adipocyte differentiation, also called adipogenesis), enlargement of adipocyte (lipogenesis), and/or reduction of lipolysis that contribute to fat accumulation in WAT. Adipocyte differentiation is defined as the formation of new adipocytes from multipotent stem cells or preadipocyte precursors [3]. In brief, adipogenesis is divided into four steps, including initial growth arrest, mitotic clonal expansion (MCE), early differentiation, and terminal differentiation-development of mature adipocyte phenotype [4]. White and brown adipocyte differentiation share common pathways and possess specific characteristics. Unless specified, "adipocyte differentiation or adipogenesis" indicates white adipocyte differentiation here.

2. Redox state changes under obese condition and in the process of adipogenesis

It is viewed that obesity is a state of chronic oxidative stress. In obese patients, increased oxidative metabolic products of DNA, protein and lipid, and/or decreased antioxidant activity are closely associated with excessive fat accumulation. In high fat (HF) diet-fed animals, oxidative stress precedes the onset of insulin resistance and obesity. In obese animals, levels of reactive oxygen species (ROS) in mature adipocytes are higher than that in other tissues, including liver, skeletal muscle, and aorta. In the course of adipogenesis, intracellular redox potentials tend to a significant oxidizing state. It is suggested that increased ROS level is associated with the development of obesity and may be required for the formation of new adipocytes.

2.1. ROS and adipogenesis

ROS plays a controversial role in adipogenesis. On the one hand, ROS is a required signal molecule in the activation of key transcription factors responsible for adipogenesis. On the other hand, ROS may also act as a negative regulator of the formation of adipocytes.

2.2. ROS is a signal required for adipogenesis

It is originally identified that ROS is produced physiologically during adipogenesis. In the last decades, accumulating data prove that endogenously generated ROS acts as an essential mediator for adipogenic differentiation. Compared with that of preadipocytes, adipocytes have a relatively higher rate of spontaneous intra- and extracellular ROS production. Adipogenic hormonal cocktails could increase ROS production in preaipocytes and enhance the process of differentiation. C/EBPβ and PPARγ are the main factors that mediate the proadipogenic effect of ROS.

2.3. ROS also functions as a detrimental signal of adipogenesis

It is shown that oxidative stress inhibits fat cell formation by reducing the C/EBP DNA-binding activity [5]. Mitochondrial ROS could inhibit preadipocyte proliferation, indicating that it can influence adipocyte differentiation through inhibiting MCE. In 3T3-F442A

preadipocytes treated with various pharmacological inhibitors, Carriere et al. [6] demonstrated that mitochondrial ROS was negatively correlated with adipocyte differentiation through increasing adipogenic repressor CHOP-10/GADD153.

2.4. ROS-producing systems and adipocyte differentiation

In a cell, ROS are mainly generated by mitochondrial respiration system, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, endoplasmic reticulum (ER), and certain metabolic and detoxifying enzymes. Effect of ROS-producing systems on adipocyte differentiation is shown in **Figure 3**.

3. Mitochondria and adipocyte differentiation

Approximate 1–3% of O_2 consumed in a cell is incompletely metabolized and diverted into superoxide in complex I and complex III in the respiratory chain. ROS production in mitochondria is obligatory during mitochondrial oxidative metabolism. In addition, ROS can also be produced by several other enzymes that locate in mitochondria. It is demonstrated that most of mitochondrial alterations is associated with redox oscillation.

Mitochondria are highly dynamic organelles that can be rapidly restructured to meet the metabolic demands timely. Notable cytoplasmic remodeling occurs during the process of adipocyte differentiation, and reorganization of mitochondrial network is one of the main events. In preadipocytes, mitochondria are filamentous and organized as continuous reticulum. In contrast, in differentiated adipocytes, they are fragmented, punctual, and redistributed around lipid droplets. Mitochondrial fusion and fission have direct influence on lipid accumulation in adipocytes. Induction of mitochondrial fusion by silencing of mitochondrial fission proteins, including dynamic-related protein (Drp1) and fission 1 homologue protein (Fis1), decreases cellular TG content. In contrast, silencing mitochondrial fusion proteins, including mitofusin 2 (Mfn2) and optic atrophy-1 (OPA1), increased cellular TG content followed by fragmentation of mitochondria.

The subunits of mitochondrial respiratory complexes I–IV increase as the differentiation progressed. There is a significant "burst" of expression level of proteins involved in the tricarboxylic acid (TCA) cycle, especially pyruvate carboxylase, at the very beginning of differentiation, which was followed by a more gradual and less intensive increase in the expression of genes associated with the electron transport chain, fatty acid metabolism, and mitochondrial transport. Above results indicate that during the transition from preadipocytes to adipocytes, cells enter a state of metabolic-overdrive characterized by simultaneous increase of flux through the TCA cycle and fatty acid oxidation.

Besides the depictive studies, several genetic and pharmacological treatments have been introduced to evaluate the role of mitochondria in adipogenesis. Depletion of mtDNA using ethidium bromide inhibits lipid accumulation in 3T3-L1 adipocytes. Carbonyl-cyanide-trifluoromethoxy-phenyl-hydrazone, an uncoupler of oxidative phosphorylation (OXPHOS),

suppresses adipogenesis. Inhibition of electron transmission chain by several agents, such as rotenone, suppresses adipogenesis by modulating the expression of the key transcription factors C/EBPα, PPARγ, and sterol regulatory element binding protein (SREBP)-1c. Several factors critical for mitochondrial function have been reported to be required for adipocyte differentiation, such as CR6-interacting factor 1 and prohibitin.

Although specific changes of mitochondria during adipocyte differentiation are variant in different laboratories, we still could depict the common pattern: mitochondrial fission, replication, and metabolism are transiently enhanced by adipogenic stimuli, and then the drastic transformation gradually "calms down" when the adipocytes are terminally differentiated. As an essential signal or by-products, enhancement of mitochondrial activity and boost of OXPHOS may result in more ROS production. In addition, direct evidence support that ROS-generating enzymes in mitochondria were found to be activated during adipocyte differentiation, such as monoamine oxidases and dihydrolipoamide dehydrogenase. Moreover, $p66^{Shc}$, a main isoform of SHC-transforming protein 1 (SHC1) that locates in mitochondria and functions as a potent redox regulator through production of ROS, could be activated by insulin, a key component of DMI cocktail. After the MCE phase, $p66^{Shc}$ phosphorylation is reduced in differentiated adipocytes.$p66^{Shc}$and consequent mitochondrial ROS generation is also implicated in the adipogenic conversion of muscle satellite cells (SCs), forming intermuscular adipose tissue. Despite the complexity of the involvement of mitochondrial alterations in adipocyte differentiation, there is a common view that mitochondria may function to influence the process of differentiation, at least, through the following ways. First, increased TCA and fatty acid oxidation meet the energy need. Second, mitochondria can provide key substrates necessary to support the massive lipogenesis during adipogenesis.

3.1. Endoplasmic reticulum and adipocyte differentiation

Endoplasmic reticulum (ER) plays an important role in regulating redox homeostasis. An exclusive oxidizing-folding environment exists in ER, which facilitates disulfide bond formation and generally produces approximately 25% of ROS in a cell. ROS could be generated in ER by uncoupling protein reaction (UPR), NADPH oxidase (NOX) 4, and microsomal monooxygenase (MMO) system.

The changes of the ER environment for protein-folding can cause the accumulation of misfolded or unfolded proteins and activate the uncoupling protein reaction (UPR), a condition also called ER stress. ER stress is a pathophysiological characteristic of obesity and exhibits notable impact on adipogenesis. On the one hand, activation of ER stress could repress adipocyte differentiation. On the other hand, UPR pathways are found to be required for adipogenic differentiation. Eukaryotic initiation factor 2 α (eIF2α)-CHOP pathway may explain the inhibitory effect of ER stress on adipogenesis. In contrast, the inositol-requiring enzyme 1 alpha/X-box binding protein 1 (IRE1alpha/XBP1) pathway of the UPR is required for adipogenesis. In addition, glucose-regulated protein 78 (GRP78), an important molecular chaperone in ER, is reported to play an essential role in adipogenesis. Protein kinase RNA-like ER kinase (PERK) utilizes its intrinsic lipid kinase activity to generate phosphatidic acid, mediates Akt activation, and promotes adipocyte differentiation. PERK can promote adipocyte differentiation

through activation of Akt. Activating transcription factor 6 (ATF6) α pathway is also involved in adipogenesis. Thus the three arms of UPR-mediated pathways, PERK, XBP1, and ATF6, are involved in adipogenesis, and blocking of ER stress prevents adipocyte differentiation and weight gain in mice. Regarding the inevitable ROS production in ER stress, the dual roles of ER stress in adipogenic differentiation could be attributed to different signal pathways, such as eIF2α-CHOP versus PERK, XBP1, and ATF6, which may be differentially regulated by ROS. Further studies are needed to test this hypothesis.

3.2. NADPH oxidases and adipocyte differentiation

The NADPH oxidases (NOXs) are important enzymatic sources of ROS in a cell. NOXs family members include catalytic subunits, known as NOX1 to NOX5, Duox1, and Duox2, and interacting partners, such as $p22^{phox}$, $p47^{phox}$, and $p67^{phox}$. Among those NOXs, only NOX4 and $p47^{phox}$ subunits have been observed to be associated with adipocyte differentiation. Role of NOX4 in adipogenesis is controversial. Compared with preadipocytes, both white and brown adipocytes possess lower expression of NOX4. In adipose tissue in vivo, NOX4 is mainly expressed in cells localized within SVF rather than mature adipocytes accumulated area. In apolipoprotein E knockout ($apoE^{-/-}$) mice, AT2 receptor deficiency-attenuated adipocyte differentiation is accompanied by an increase in NOX activity. In this trend, NOX4-deficient mice display latent adipose tissue accumulation and are susceptible to diet-induced obesity and early onset of insulin resistance, which could be attributed to accelerated adipocyte differentiation and hypertrophy. Above evidence suggests that NOX4 acts as a negative regulator of adipocyte differentiation.

However, opposite data have been reported. In hypertrophied adipose tissue, ROS level is increased accompanied by an increase of NOX. In 3T3-L1 adipocytes, NOX4 is required for proper insulin signaling transduction through ROS generation. Thus, pharmacological inhibition of NOX4 or KD of NOX4 by RNA interference inhibits ROS production and adipocyte differentiation. As a component of the NOX, $p47^{phox}$ is shown to be positively related with adipocyte differentiation. Mice lacking $p47^{phox}$ are protected against HF diet-induced increase of adiposity, adipocyte size, and hepatic and alcohol-induced hepatic steatosis. Whether other NOX isoforms are involved in adipogenesis is currently unclear.

3.3. Xanthine oxidoreductase and adipocyte differentiation

In some cases, activation of xanthine oxidoreductase (XOR) contributes to ROS generation through the enzymatic form of xanthine dehydrogenase (XDH) or xanthine oxidase (XO). It is reported that XOR colocalizes with the lipid-binding protein adipophilinon the milk-fat globule, functioning as a major component of the milk-fat globule membrane, and is required for fat-droplet secretion in mice. Cheung et al. first show that XOR is highly expressed in adipose tissue compared to other tissues and is transiently induced during 3T3-L1 adipocyte differentiation in vitro. Under obese state, XOR expression is increased in the adipose tissue. In vivo, adipose mass is reduced by 50% in $XOR^{-/-}$ mice versus wild-type littermates. KD of XOR inhibits PPARγ activity and adipogenesis in vitro. The deficiency of adipogenesis in cells with XOR down-regulation is fully restored by an addition of rosiglitazone, implicating that

XOR is at the upstream of PPARγ. However, constitutive overexpression of XOR increases PPARγ activity but inhibits adipogenesis. The authors explain that XOR may be transiently required for adipogenic differentiation, and thus prolonged expression of XOR may exert unexpected results. In addition, C/EBPβ is shown to transcriptionally regulate XOR level in several cell types. These results support the notion that XOR lies downstream of C/EBPβ and upstream of PPARγ in the cascade of factors that control adipogenesis and is required for adipocyte differentiation. Moreover, although lacking direct evidence, the finding that inhibition of XOR's dehydrogenase activity suppresses PPARγ activation and inhibits adipogenesis, indicates that NADH-oxidizing activity of XOR and the concomitant generation of ROS might be involved in adipogenic regulatory role of XOR.

3.4. Nitric oxide synthases and adipocyte differentiation

NO, a ubiquitous signaling molecule belonging to reactive nitrogen species (RNS), is synthesized by nitric oxide synthases (NOS). There are three isoforms of NOS, including neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). NO is found to promote differentiation of rat brown and white preadipocytes. The mechanism of NO-promoted adipogenesis involves the activation of guanylyl cyclase and increase of cGMP, which in turn up-regulates cAMP and leads to enhancement of adipogenesis. In addition, NO is shown to induce mitochondrial biogenesis in brown adipocytes, which is dependent on induction of PGC-1α expression.

Both eNOS and iNOS exist in adipose tissue. iNOS expression is increased in adipogenic differentiation. Clinical studies have suggested that increase of NOS and NO level strongly correlate with body fat in obese humans, and eNOS expression is increased in omental versus subcutaneous adipose tissue in obese human subjects. In contrast, it is also found that eNOS levels are reduced in the adipose tissue of obese humans, db/db mice, and HF diet-fed mice. Moreover, overexpression of eNOS prevents HF diet-induced obesity and attenuates hypertrophy of WAT. It is more likely that iNOS and eNOS play differential roles in adipocyte differentiation through regulating NO generation under different conditions. Inducible NO generation by iNOS may be a required event for adipogenic differentiation.

3.5. Cytochrome P450 and adipocyte differentiation

The cytochrome P450 enzymes (CYPs) is a major source of ROS in various tissues and cells. AA is metabolized by several CYP isoforms to produce 20-HETE and epoxyeicosatrienoic acids (EETs). It has been shown that 20-HETE promotes ROS production, whereas EETs is associated with inhibition of ROS production. Under obese condition, CYPs family could be altered in a complicated and isozyme-specific way. CYP3A4 is decreased and CYP2E1 is increased in obesity. The changing patterns of CYP1A2, 2C9, 2C19, and 2D6 isozymes under obese condition are inconclusive. In addition, CYP2E1 expression and activity are increased in female ob/ob mice, while that of CYP1A2 are decreased in male ob/ob mice regardless of gender. Inhibition of CYP27A1 activity, or KD and/or deletion of the Cyp27a1 gene induce adipocyte differentiation through regulating oxysterol 27-hydroxycholesterol. During the differentiation of MSCs to adipocytes, CYP4A11 and CYP4F2 expression are decreased to nearly undetectable level. CYP2J5 is decreased in visceral adipose tissue isolated from HO-2 null mice, in which adipocyte differentiation is enhanced.

3.6. Antioxidant enzymes and adipocyte differentiation

The enzymatic antioxidants are capable of counteracting ROS/RNS and oxidant insults through catalyzing a variety of redox reactions.

3.7. Superoxide dismutase and adipocyte differentiation

Regarding the dual roles of superoxide dismutase (SOD) in eliminating superoxide and generating hydrogen peroxide, SOD may play opposite roles in adipocyte differentiation. It is reported that SOD1 expression is decreased in adipose tissue of obese mice. However, in $Sod2^{-/-}$ BMSCs, adipogenesis is found to be increased. Amifostine, an antioxidant, was shown to decrease the numbers of adipocytes in $Sod2^{-/-}$ BMSCs in both basal and adipogenic media and reduce the expression of PPARγ and LPL. In contrast, in $Sod2^{+/+}$ BMSCs, adipogenesis was decreased in adipogenic medium. During 3T3-L1 adipocyte differentiation, SOD expression and activity were also found to be increased. In mice fed with HF diet, MnTBAP, a pharmacological analogue of SOD, attenuates weight gain and adiposity through a reduction in adipocyte hypertrophy, adipogenesis, and fatty acid uptake in epididymal (eWAT) but not in inguinal (iWAT) adipose tissue.

3.8. Catalase and adipocyte differentiation

Catalase (CAT) catalyzes the decomposition of hydrogen peroxide to water and oxygen. mRNA expression of CAT is decreased in the adipose tissue in obese mice. The decreased CAT activity resulting from its promoter mutation is positively associated with childhood obesity and obesity biomarkers such as weight, BMI, and aP2, along with a tendency toward significance with insulin resistance biomarkers. However, in inguinal WAT in obese Zucker rats, CAT activities were found to be increased significantly. Similarly, CAT expression and activity are greatly elevated along with the progression of 3T3-L1 adipocyte differentiation. Furthermore, a remote enhancer region containing two functional PPARγ binding sites in mouse CAT gene was found, suggesting that increased CAT expression during adipocyte differentiation may be a subsequent consequence of PPARγ activation. Thus, whether there is a causal role of CAT alteration in adipocyte differentiation is still unelucidated.

3.9. Thiol-dependent antioxidant system and adipocyte differentiation

Thiol-dependent antioxidant system mainly consists of glutathione (GSH), thioredoxins (Trxs), and peroxiredoxins (Prxs). The interactions between these antioxidant proteins form a potent antioxidant defense system against potential oxidant threat.

3.10. Glutathione metabolic system and adipocyte differentiation

GSH, also named γ-L-glutamyl-L-cysteinyl-glycine, is the most important hydrophilic antioxidant. Glutamate-cysteine ligase (GCL) is the rate-limiting enzyme for de novo synthesis of GSH from the precursor amino acids cysteine, glutamate, and glycine in cooperation with glutathione synthase (GS). GCL consists of a modulatory or light subunit (GCLm) and a catalytic or heavy subunit (GCLc). GSH could eliminate oxidant radicals through nonenzymatic

reaction. GSH-mediated reduction of hydroperoxides needs the enzymatic catalysis by GSH peroxidase (GPx). Glutathione-S-transferases (GSTs) detoxify xenobiotic compounds through catalyzing the conjugation of GSH to nonpolar substrates. After the reduction of oxidants or the detoxification of toxicants, GSH form GSSG (oxidized form of GSH) or GSSR (glutathionylated-cysteine derivative), which can be reduced back to GSH catalyzed by NADPH-dependent GSH reductase (GR) and Trxsystem.

3.11. General GSH status and adipocyte differentiation

Compared with WT littermates, GSH level in adipose tissue is higher in ob/ob mice. Sulfhydryl depletor DEM significantly inhibits adipogenesis, suggesting GSH is involved in the adipogenic process. Moreover, in vivo evidence shows that pharmacological depletion of the GSH in mice prevents diet-induced obesity and increases energy expenditure.

However, controversial data have been reported that up-regulation of GSH was involved in carnosic acid (CA) and carnosol (CS)-inhibited adipocyte differentiation. As a precursor of cysteine, intracellular NAC can be converted into GSH. NAC can be used for the treatment of acetaminophen-induced hepatic injury and for the prevention of radio contrast-induced nephropathy due to its potent antioxidant property. NAC dose-dependently inhibits ROS level and attenuates DMI-induced adipogenesis in 3T3-L1 cells, accompanied by inhibited expression of C/EBPβ and PPARγ. NAC also blocks adipocyte differentiation and the transcriptional activation of CREB in MSC. During ST2 cell adipogenesis, the intracellular GSH redox potential becomes more oxidized. Furthermore, intraperitoneal administration of NAC to rats and mice resulted in reduction of bodyweight. These data suggest that dysregulation of GSH may disturb the physiological role of GSH redox in adipogenic differentiation.

3.12. GCL and adipocyte differentiation

GCL expression in adipose tissue is higher in ob/ob mice in comparison to WT littermates. BSO, a specific and irreversible inhibitor of GCL, deletes GSH and inhibits the differentiation of 3T3-L1preadipocytes and preadipocytes derived from the SVF of inguinal as well as epididymal fat pads. BSO inhibits adipocyte differentiation through inhibition of MCE via down-regulation of E2F-dependent transactivation of the MCM7 target promoter. However, up-regulation of GCLc was proposed to be involved in CA and CS-inhibited adipocyte differentiation.

3.13. GPx and adipocyte differentiation

GPx1, 3, 4, and 7 are identified to be abundantly expressed in mature adipocytes and WAT. In parallel with lipid accumulation during adipocyte differentiation, GPx activity is increased. However, controversial data are also reported.

Compared with WT counterparts, mice over-expressing GPx1 develop hyperglycemia, hyperinsulinemia, and obesity. In patients with nonalcoholic fatty liver disease (NAFLD), the increase in GPx1 expression level is an independent variable associated with NAFLD progression. Chung et al. suggest that GPx3 is required for the regulation of PPARγ-mediated antioxidant effects. Although GPx3 is abundantly expressed in adipose tissues, its expression is reduced

selectively in the adipose tissue of several obese animal models. Antioxidant NAC and the anti-diabetic drug rosiglitazone increase adipose GPx3 expression in obese and diabetic db/db mice.

GPx4 is stimulated in the course of 3T3-L1 adipocyte differentiation. GPx7 is highly enriched in WAT and is mainly expressed in preadipocytes but not mature adipocytes of WAT. In response to induction medium, GPx7 level in 3T3-L1 preadipocytes declines rapidly within the first 24 h. GPx7 deficiency in 3T3-L1 preadipocytes increases the expression of key transcription factors involved in adipogenesis, including C/EBPβ, C/EBPα, and PPARγ. GPx7 deficiency increases C/EBPβ expression through PKA signaling pathway in a ROS-dependent manner. In addition, deficiency of GPx7 promoters C/EBPβ dimerization increases its binding to the promoter of C/EBPα, leading to an increase of cell proliferation and expression of downstream targets C/EBPα and PPARγ. Furthermore, human genetic variant association results suggest that the subjects carrying risky GPx7 alleles may have reduced GPx7 expression in adipose tissue and higher BMI. However, GPx7 level was increased in the late period of adipocyte differentiation in human liposarcoma cell line SW872.

3.14. GST and adipocyte differentiation

GSTA3 is over-expressed at the late stage of adipogenesis in human liposarcoma cell line SW872. Jowsey et al. identified GSTA3 as a novel adipocyte differentiation-associated protein whose expression was enhanced manyfolds during the conversion of mouse preadipocytes into adipocytes. During the terminal phase of adipogenesis in 3T3-L1 preadipocytes, GSTζ is found to be increased in C/EBPα- and PPARγ-dependent manner. However, in diet-induced obese rats, GSTYc2 subunit, GST8, and GSTP subunits were found to be down-regulated. Up-regulation of GSTA2 may participate in CA and CS-inhibited adipocyte differentiation.

3.15. The thioredoxins system and adipocyte differentiation

The Trxs system is composed of NADPH, Trx, thioredoxin reductase (TrxR), thioredoxin interacting protein (Txnip), and novel nucleoredoxin (Nrx). TrxR reduces oxidized Trx. TXNIP is a negative regulator of Trx, resulting in oxidative stress. There are significant alterations of Trx system during adipogenesis. Rajalin et al. showed that the protein level of TrxR1, TrxR2, and Trx2 was elevated in the course of 3T3-L1 adipocyte differentiation. Chutkow et al. found that Txnip protein dramatically disappeared within minutes after DMI stimulation, indicating that Txnip degradation is required for the onset of adipocyte differentiation. Over-expression of Txnip in preadipocytes prevents adipocyte differentiation, whereas its down-regulation enhances adipogenesis. Compared with WT littermates, Txnip KO mice gain significantly more adipose mass due to calorie intake and adipogenesis. In Txnip-silenced preadipocytes and Txnip$^{-/-}$ mouse embryonic fibroblasts (MEFs), adipogenesis is markedly increased, whereas Txnip over-expression impaired adipocyte differentiation. Txnip deletion could also augment PPARγ-stimulated adipogenesis. Txnip negatively regulates the expression and activation of PPARγ, which in turn, suppresses Txnip expression, reflecting reciprocal feedback inhibition between them. In 3T3-L1 preadipocytes, Trx over-expression stabilized Txnip protein levels and thus promoted inhibition of adipogenesis. As an important component of adipogenic stimulants, insulin promoted the dissociation of Txnip-Trx into a more labile state.

Nrx level is higher in WAT of ob/ob mice and is increased in the early adipogenic stage of 3T3-L1 preadipocyte differentiation. KD of Nrx decreases and its over-expression increases adipogenic differentiation of 3T3-L1 cells. In transgenic mice with adipose tissue-specific Nrx over-expression, adipocyte size as well as number is increased compared with WT mice. Moreover, inhibition of Wnt/β-catenin pathway is involved in Nrx-exerted regulation of adipogenic differentiation.

3.16. Peroxiredoxins and adipocyte differentiation

As one of the most abundant antioxidant proteins, Prxs are able to reduce hydrogen peroxide and organic hydroperoxides to water and alcohol. Genetic variations of Prx3 are associated with the risk of obesity. In WAT of db/db mice and in perirenal WAT of human subjects with BMI > 25, Prx3 expression is significantly decreased. Huh et al. first studied the role of Prxs in adipocyte differentiation in detail and showed a gradual increase inPrx1, Prx3, and Prx5 expression in the course of 3T3-L1 adipocyte differentiation. Compared with Prx1 and Prx5, Prx3 was increased by a larger extent. At the age of 20 months, Prx3 KO mice display larger fat pads in epididymal WAT with hypertrophied adipocytes. In differentiated ADSCs, expression of aP2, C/EBPα, and PPARγ, adipophilin, and FAS are increased in Prx3 KO mice. In 3T3-L1 preadipocytes, siRNA-mediated KD of Prx3 increased aP2, C/EBPα, and PPARγ expression, whereas Prx3 over-expression inhibited their expression. Moreover, ROS level in mitochondria was increased in WAT of Prx3 KO mice. In MSCs, over-expression of Prx2 also shows inhibitory effect on the lipid accumulation during adipocyte differentiation.

3.17. NADPH: quinone oxidoreductase 1 and adipocyte differentiation

NADPH:quinone oxidoreductase 1 (NQO1) may generate quinones, quinoneimines, and andazodyes accompanied by inhibition of ROS generation. NQO1 protein is present in human adipocytes, and NQO1 mRNA level is higher in adipocytes than in adipose-derived stromal vascular cells. NQO1 expression is positively correlated with BMI and weight loss results in a decrease in NQO1 mRNA content in adipose tissue. In comparison to WT mice, $NQO1^{-/-}$ mice exhibit lower abdominal adipose tissue. Using 3T3-L1 preadipocytes, Vomhof-DeKrey et al. investigated NQO1 expression in the course of adipocyte differentiation. They found an increase of NQO1 protein at limited MCE and postmitotic growth arrest (Days 1–3) stages and a decrease in terminally differentiated (Day 8) adipocytes that lasted for several days afterward. In contrast to the mapping of protein, NQO1 mRNA expression was increased in differentiated adipocytes (Days 11–14), indicating a discrepancy between steady-state of mRNA and resulting protein levels. Sulforaphane (SFN) enhanced NQO1 protein level and blunted TG accumulation in a NQO1-dependent manner.

3.18. Heme oxygenase and adipocyte differentiation

Heme oxygenase or haem oxygenase (HO) is an enzyme that catalyzes the degradation of heme and produces free iron, biliverdin, and carbon monoxide. Three isozymes of HO have been defined, including inducible heme oxygenase-1 (HO-1), constitutive heme oxygenase-2(HO-2) and not fully defined HO-3. HO-1 exhibits antioxidant capacity due to its products

bilirubin/biliverdin and carbon monoxide. The HO system acts as a key cellular antioxidant defense system in obesity and diabetes. Decreased HO-1 expression levels were observed in type 2 diabetic patients. In addition, products of HO-1 catalysis, CO, and bilirubin are decreased in humans and animal models of type 2 diabetes. Zucker fat (ZF) rats display a decrease in both HO activity and HO-1 and HO-2 protein expression.

A number of studies have demonstrated that genetic inhibition of HO expression and activity increase adipogenesis. In vitro induction of HO-1 decreases adipogenesis. siRNA-mediated inhibition of either HO-2 or HO-1 leads to an increase in adipogenesis. Up-regulation of HO-1 induces a decrease of adipocyte differentiation in MSCs. BMSCs from HO-$2^{-/-}$ mice displayed an increase in adipogenesis and accumulation of lipid droplets, resulting in adipocyte hypertrophy. Using a lentivirus construct of the human HO-1 under the control of theaP2 promoter, Cao et al. revealed that adipocyte-specific over-expression of HO-1 attenuated adiposity and adipogenesis.

In addition, pharmacological studies support the notion that HO-1 negatively regulates adipocyte differentiation. Cobalt protoporphyrin (CoPP) or L-4F, a systemic apomimetic peptide inducer of HO-1, promotes chronic body weight loss in various species, decreases visceral and subcutaneous fat content, and reduces the number of enlarged adipocytes, and increases adiponectin levels and small adipocytes, without any effect on food intake, or other metabolic changes such as energy expenditure and O_2 consumption. In addition, other HO-1 inducers, such as D-4F, an apolipoprotein A1 mimetic peptide, replicate the body weight-lowering effect of HO-1 induction. In ZF animals and human bone marrow-derived adipocytes, HO-1 expression and activity was found to be increased by CoPP treatment. In vivo, HO-1 up-regulation was associated with decrease in superoxide levels, visceral and subcutaneous fat content and weight gain, and increase in plasma adiponectin. In vitro, up-regulation of HO-1 decreased superoxide level, adipose remodeling, smaller adipocytes, and increased adiponectin secretion in the culture medium. Burgess et al. found that induction of HO-1 by CoPP slowed the rate of weight gain in male obese mice and produced a significant decrease of IL-6, TNFα, and IL-1β. In B6v-Lep obese/J mice, HO-1 induction increases the number, whereas decreases the size of adipocytes. Moreover, the chronic treatment of carbon monoxide, the product of HO metabolism, prevents the development of HF diet-induced obesity via stimulating metabolism and remodeling adipocytes. In addition, over-expression of HO-1 increases pAMPK and eNOS levels and promotes human osteoblastic differentiation, another direction of cell differentiation. It was found that induction of HO-1 was associated with reduction of C/EBPα, PPARγ, Peg-1/Mest, aP2, CD36, Wnt5b expression and the increase of β-catenin, pGSK3β, Wnt10b, Pref-1, shh, and adiponectin, indicating that those key factors were responsible for HO-exerted effect on adipogenesis.

However, Huang et al. reported that over-expression of HO-1 did not protect against HF diet-induced body weight gain and insulin resistance in mice. Vanella et al. reported that L-4F increased early adipocyte differentiation markers and decreased Peg-1/Mest through activation of HO-1. In human MSCs, HO-2 depletion results in increase of adipogenesis and inflammatory cytokines, with lower expression of HO-1. Sofalcone, a chalcone derivative, inhibits the differentiation of 3T3-F442A preadipocytes into adipocytes, which is restored by SnPP treatment, indicating the involvement of HO-1.

3.19. Glucose-6-phosphate dehydrogenase and adipocyte differentiation

Glucose-6-phosphate dehydrogenase (G6PD) generates NADPH, providing the substrate for NOX and reducing equivalent for GSH and Trx system. Thus, G6PD could affect ROS level in a bidirectional manner. NADPH is also required for the biosynthesis of fatty acids and cholesterol. The expression and enzymatic activity of G6PD are significantly elevated in WAT of obese models, including db/db, ob/ob, and diet-induced obese mice. In 3T3-L1 cells, G6PD overexpression stimulates the expression of most adipocyte markers and elevates the levels of cellular FFA, triglyceride, and FFA release, indicating an important role of G6PD in adipogenesis.

3.20. Selenoproteins and adipocyte differentiation

Selenoprotein P (SeP), a circulating selenium carrier, functions as an antioxidant enzyme. Although it is expressed most abundantly in liver, SeP also plays a role in the regulation of lipid metabolism in adipocyte through redox modulation. Zhang et al. reported that SeP1 gene expression was significantly reduced in adipose tissue of ob/ob and HF diet-induced obese mice as well as in primary adipocytes isolated from ZF rats. SeP expression is induced in the course of 3T3-L1 adipocyte differentiation. In adipose tissue of obese mice, rosiglitazone treatment increased SeP protein expression. In contrast, exposure to either TNFα or high level of H_2O_2 reduces SeP1 expression in a time- and dose-dependent manner in differentiated 3T3-L1 adipocytes. Moreover, KD of SeP1 reduces GPx activity, whereas increases the expression inflammatory cytokines, such as MCP-1 and IL-6 in preadipocytes. These characteristics contribute to the inductive role of SeP in 3T3-L1 adipocyte differentiation.

3.21. Metallothioneins and adipocyte differentiation

Metallothioneins (MTs) are a family of proteins with abundant cysteine residues. MTs are capable of regulating oxygen respiration, possessing potential antioxidant activities. MTs function as regulators of various cellular processes including gene expression, apoptosis, proliferation, and differentiation. Evidences suggest that MTs are altered during adipocyte differentiation and play important roles in regulation of adipocyte differentiation. In human, metallothionein-2A (MT-2A) mRNA level in fat tissues is significantly elevated in obese subjects. MT-2 expression is higher in omental than in subcutaneous adipose tissue. Moreover, MT could be secreted by fat cells in WAT. However, Sato et al. discovered that $MT^{-/-}$ mice fed with a HF diet exhibited more fat mass and a larger adipocyte volume, indicating that MT may have a preventive role against HF diet-induced obesity.

3.22. Non-enzymatic antioxidants and adipocyte differentiation

In addition to those enzymatic antioxidants, endogenous and exogenous nonenzymatic antioxidants also play important roles in regulating lipid homeostasis. They are usually classified into water-soluble antioxidants, including GSH, vitamin C (V_C), lipoic acid (LA), and uric acid (UA), or lipid-soluble antioxidants, such as β-carotene, vitamin E (V_E), and coenzyme Q (CoQ). The adipose tissue of ob/ob mice exhibits higher content of hydrophilic molecules (GSH, V_C) in a lower redox state, which is associated with lower content of lipophilic

molecule (V_E, CoQ) and lipid peroxidation. In particular, CoQ is deficient in WAT of ob/ob mice. In rodents as well as in humans, CoQ content is strongly and negatively correlated with subcutaneous adipose tissue and obesity indexes. In 3T3-F442A cell line, pharmacological inhibition of CoQ synthesis by chlorobenzoic acid strongly triggers adipose differentiation. In contrast, over-expression of 4-hydroxybenzoate acid polyprenyltransferase increases CoQ level and inhibits adipogenesis of 3T3-F442A cell. These data suggest that CoQ is an anti-adipogenic factor. NADPH is an essential reducing equivalent for numerous enzymes. Cytosolic $NADP^+$-dependent isocitrate dehydrogenase (IDPc) is a key enzyme for providing cytosolic NADPH. In 3T3-L1 preadipocytes and mice, KD of IDPc reduces adipogenesis and lipid accumulation.

3.23. Antioxidant extracts/natural products and adipocyte differentiation

In the last decades, dietary supplementation of natural antioxidants is prevalent worldwide. The dramatic discrepancies about the biological outcomes of antioxidant consumption have been reported.

A large number of experimental and clinical investigations have shown that many antioxidants possess potent anti-obesity effects. α-lipoic acid, a famous ROS scavenger for hydroxyl radicals and singlet oxygen, exerts reproducible anti-obesity effects in several independent clinical trials. The inhibition of adipocyte differentiation by α-lipoic acid involves the regulation of pro-adipogenic transcription factors via MAPK pathways. Dietary orlistat supplementation induces a reduction of body weight in rats fed with HF diet. Bardoxolone methyl, a novel antioxidant capable of activating the Keap1-Nrf2 pathway, shows unexpected weight-reducing effects in a recent clinical trial. Brazilein, a natural biologically active compound derived from *Caesalpinia sappan* L., exhibits antioxidant properties and inhibits adipocyte differentiation. Combined treatment of mulberry leaf and fruit extract ameliorates HF diet-induced obesity in mice. Diet supplemented with 5% ginger powder reduces body weight of rats fed with HF diet. *Grateloupia lanceolata* (Okamura) Kawaguchi, the edible red seaweed, was found to inhibit lipid accumulation and ROS production during 3T3-L1 cell differentiation. Tempol could attenuate adipogenesis in both hMSCs and 3T3-L1 cells. Ethyl acetate extract of ginger, the rhizome of *Zingiber officinale* Roscoe, possesses antioxidant capacity and inhibits adipocyte differentiation. Dietary ascorbic acid was able to protect against HF diet-induced increase of body weight and total body fat, and enlargement of different adipose depots, without affecting food intake. Resveratrol and its analogues, (E)-1,2-di (3,5-dimethoxyphenyl)ethane and 4-[2-(3,5-dimethoxyphenyl)vinyl] pyridine, inhibit adipocyte differentiation in 3T3-L1 cells via activation of AMPK. Oleanolic acid is a triterpenoid compound that has potent antioxidative and anti-inflammatory properties. In differentiated 3T3-L1 adipocytes, oleanolic acid was shown to decrease lipid accumulation and the expression level of differentiation markers, including PPARγ and C/EBPα. Pycnogenol® is a group of flavonoids with antioxidative effects. In 3T3-L1 adipocytes, Pycnogenol® inhibits mRNA expression of NOX4, G6PD, PPARγ, and C/EBPα, whereas increases the level of SOD1, SOD2, GPx, and GR. *Polygonum aviculare* L. ethanol extract exhibits anti-diabetic effect in HF diet-induced obese mice. Buckwheat sprouts exhibits anti-adipogenic and anti-oxidative properties.

Phenolic compounds, which are secondary plant products, are consumed regularly as part of the human diet and are helpful for disease prevention. Oleuropein is the principal phenolic compound in olive tree products, which is the main source of healthy Mediterranean diet ingredients. Hydroxytyrosol is a bioactive substance after the hydrolysis of oleuropein. It is reported that oleuropein and hydroxytyrosol exhibit antioxidant activities and inhibit adipogenesis in stem cells derived from human bone marrow and in 3T3-L1 preadipocytes. In 3T3-L1 preadipocytes, oleuropein and hydroxytyrosol dose-dependently suppressed fat accumulation and differentiation-related markers, including PPARγ, C/EBPα, SREBP-1c, GLUT4, CD36, and FASN through suppression of MCE. Cigarette smoking aggravates Graves' orbitopathy through oxidative stress-mediated adipogenesis. Quercetin, as an antioxidant, inhibits adipogenesis by reducing ROS in cultured orbital fibroblasts from Graves' orbitopathy patients treated by cigarette smoke extract.

However, there is also solid evidence supporting the conception that consumption of antioxidants promotes adipocyte differentiation and obesity. Abe et al. prepared a 100% methanol fraction of methanolic extract from unripe kiwi fruit (*Actinidia deliciosa*) and found that the extracts decreased the production of ROS and promoted adipocyte differentiation. Patients treated with clozapine often suffer from massive weight gain. Clozapine-induced increase of fat mass is the result of enhanced fat cell formation from adipogenic precursor cells, which may be attributed to antioxidant effects. Puerarin is a major isoflavone glycoside derived from Kudzu root (*Pueraria lobata*) that possesses antioxidant effects. Puerarin could enhance 3T3-L1 adipocyte differentiation, accompanied by increased expression of G6PD, and PPARγ and its target genes, and several antioxidant enzymes, including GR and CAT. Butylhydroxyanisole inhibits lipid accumulation and adipocyte differentiation in 3T3-F442A in a concentration-dependent manner.

3.24. Redox-sensitive transcriptional factors and adipocyte differentiation

Beyond the direct regulation of ROS by ROS-generating enzymes and enzymatic and non-enzymatic antioxidants, redox balance is controlled by a wide class of transcription factors, including nuclear factor erythroid 2-related factor 2 (Nrf2), peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), p53, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and forkhead box O (FoxO). These transcription factors could respond to subtle redox changes, determining the reaction toward oxidation or reduction through transcriptionally modulating ROS-generating enzymes and antioxidant enzymes. The large amount of transcription factors influencing adipocyte differentiation play particularly important roles in a cooperative and interactive way.

3.25. Nrf2 and adipocyte differentiation

Nrf2 is the most thoroughly studied redox-sensitive transcription factor. Under normal conditions, Nrf2 is inactivated by kelch-like ECH-associated protein 1 (Keap1), which targets Nrf2 for proteasomal degradation. Once activated, Nrf2 binds to antioxidant response elements (AREs) or electrophile response elements (EpREs), inducing the expression of more than 100 genes involved in the response to cellular stress. Functioning as a "supreme director" in redox

regulation, Nrf2 can regulate both ROS/RNS-generating enzymes (such as SOD and iNOS) and antioxidant enzymes. However, Nrf2 more likely acts as an antioxidant regulator in most cases. Redox proteins regulated by Nrf2 are listed as follows: NQO1, GCLc and GCLm, GPxs, GR, GSTs, HO-1, SOD, CAT, Trxs, TrxRs, Prxs, UCPs, NOS, and G6PD [7, 8].

A large number of papers have revealed contradictory roles of Nrf2 in the regulation of metabolism. On the one hand, Nrf2 is critical for antioxidant defense system, innate immunity, protection against inflammation, insulin resistance and diabetes, and cancer. On the other hand, Nrf2 deficiency is beneficial for the attenuation of atherosclerosis, HF diet-induced obesity and insulin resistance, and cancer.

The Nrf2/Keap1 pathway is linked to the development of obesity and hepatic lipid accumulation in animal models. Various natural and synthetic substances with the ability to stimulate Nrf2 have been verified to exert anti-obesity activity, including 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im), oleanolic acid, oltipraz, ellagic acid, quercetin, curcumin, resveratrol, and chromium histidinate. Keap1 KD mice with a constitutive increase in Nrf2 activity have lower body weight and epididymal fat masse than WT mice. Enhanced expression of Nrf2 in mice attenuates the fatty liver induced by special diet. However, using Nrf2 KO mice, Nrf2 deficiency has been found to protect against HF diet-induced obesity. However, no significant relationship between Nrf2 and obesity has also been reported. Tanaka et al. found that in both WT and Nrf2-null mice fed with a HF diet for up to 4 weeks, weight gain shows no difference. Consistent with its role in obesity, Nrf2 functions as a double-edged sword in the pathogenesis of insulin resistance. Studies using genetic manipulation have shown that Nrf2 deficiency is beneficial for glucose homeostasis and insulin sensitivity.

Nrf2 is highly expressed in the adipose tissue and animals fed with HF diet exhibit higher Nrf2 expression. As an important constituent of adipogenic stimuli, insulin could significantly activate Nrf2 pathway [9]. These findings suggest a potential role of Nrf2 in the modulation of adipogenesis. Indeed, genetic manipulation and pharmacological activator treatment have been introduced to investigate the role of Nrf2 in adipogenesis and lipid metabolism, which is contradictory. Role of the Nrf2/ARE pathway in energy metabolism and storage has been reviewed by Vomhof-DeKrey et al. In this review, we focus on the involvement of Nrf2/Keap1 pathway in the regulation of adipocyte differentiation.

3.26. Activation of Nrf2 promotes adipogenesis

Pi and his colleagues have made great contributions to the identification of the proadipogenic effect of Nrf2. In their studies, Nrf2 was found to be activated in the early stage (<12 h) of adipogenesis [10]. Consistent with the notion that Nrf2 is a key regulator for GSH synthesis, intracellular GSH level follows a similar pattern as Nrf2-ARE. However, in another study, Nrf2 and Keap1 mRNA levels were found to be increased in differentiated adipocytes (Days 11–14), indicating a programmed control of Nrf2 during adipogenic differentiation. Pi et al. showed that KD of Nrf2 could totally block the enhancement of adipogenesis in Keap1-KD cells, confirming the contribution of Nrf2 activation to above process. To directly determine the regulatory role of Nrf2 in adipogenesis, preadipocytes derived from WAT of Nrf2-KO ($Nrf2^{-/-}$) and WT ($Nrf2^{+/+}$) mice were used. Hormonal cocktail-induced adipogenesis in $Nrf2^{-/-}$

preadipocytes shows substantially reduced lipid accumulation. Moreover, Nrf2$^{-/-}$ preadipocytes exhibit significantly decreased expression of adipogenic genes, including PPARγ1, PPAR2, adipsin, and aP2. In 3T3-L1 preadipocytes and human subcutaneous preadipocytes, shRNA-mediated KD of Nrf2 expression inhibits adipogenesis. Pi et al. also found that mice Nrf2 deficiency displayed reduced fat mass in association with small adipocytes and increased adipocyte numbers, and were resistant to diet-induced obesity.

3.27. Activation of Nrf2 inhibits adipogenesis

In contrast to the discovery by Hou et al. [10], Shin and colleagues [11] demonstrated an inhibitory role of Nrf2 in adipogenesis using Nrf2$^{-/-}$-immortalized MEFs. In Keap1$^{-/-}$-primary MEFs, adipogenesis is inhibited by enhanced Nrf2 signaling, compared to WT MEFs. In Nrf2$^{-/-}$-immortalized MEFs, ectopic expression of dominant-positive Nrf2 delays differentiation. HF diet feeding decreased the expression of Nrf2 and its target genes, such as NQO1 and GSTm6, and Nrf2 inhibited lipid accumulation in mouse liver after feeding a HF diet. Chartoumpekis et al. found a decrease in nuclear abundance and DNA binding activity of Nrf2 during adipogenesis in ST2 cells. Vomhof-Dekrey et al. speculate that these controversial data may be mainly due to the differential use of primary versus immortalized MEFs and the different experimental periods. It is highly recommended to construct adipose tissue-specific Nrf2 knockout mice to investigate its role in adipogenesis.

3.28. Obesity and ROS, friends or foes?

From an evolutionary standpoint, obesity protects humans from potential threat of food shortage through energy storage. Adipocyte differentiation is a protective mechanism essential for survival, in which energy is stored in the form of TG in the adipose tissue. In this tendency, adipocyte differentiation is a protective mechanism essential for survival. However, this protective mechanism would lead to obesity without control, causing a series of unhealthy problems. In obese individuals, WAT release proinflammatory factors, resulting in metabolic dysfunctions [461]. Moreover, ectopic accumulation of fat in other tissues is detrimental for normal physiological functions, leading to several serious diseases.

In aerobic organisms, the capability of oxygen consumption precedes the onset of advanced life and promotes the biological evolution. Three decades ago, ROS was considered to be generated as by-products of oxygen consumption. Current concepts support that ROS may be evolutionally utilized as essential factors for the regulation of various biological processes that reflect the essence of "concise" in life activity. In the last decades, more and more evidences have shown the active role of ROS in the regulation of life processes rather than the passive role in inducing oxidative injury.

3.29. Novel hypothesis of redox system: Redox Chain

Based on the current results and the complexity of redox system, we propose that there is a complex and interactive "Redox Chain," consisting of "ROS-generating Enzyme Chain," "Combined Antioxidant Chain," and "Transcription Factor Chain" (**Figure 1**). On the oxidant

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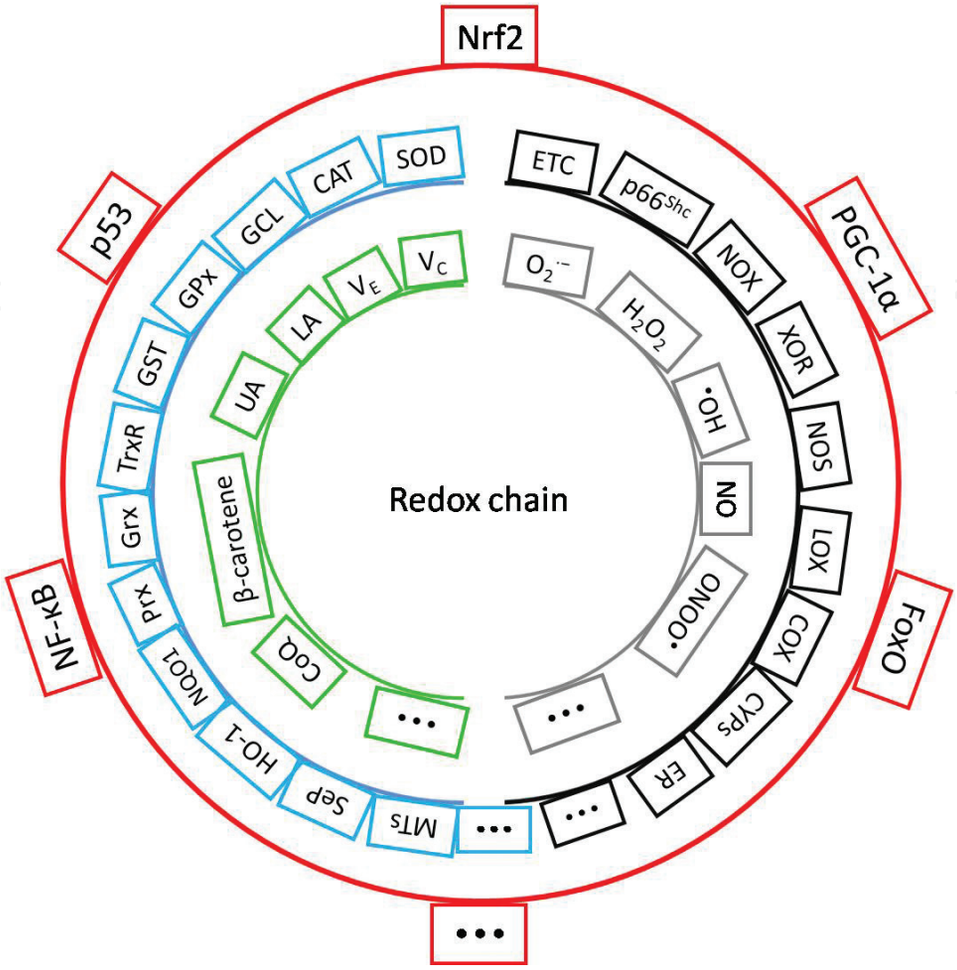

Figure 1. A hypothesis of "Redox Chain." The innermost line on the left indicates "Nonenzymatic Antioxidant Chain". The middle line on the left indicates "Antioxidant Enzyme Chain". The innermost line on the right indicates "Oxidant Chain". The middle line on the right indicates "ROS-generating Enzyme Chain". The outermost line indicates "Transcription Factor Chain".

side, "ROS-generating Enzyme Chain" consisted of enzymes that are responsible for intracellular ROS generation, including ROS-generating enzymes in mitochondria and ER, NOXs, XOR, NOS, LOX, COX, CYPs, and other undefined enzymes that may be involved in ROS production. In response to physiological and/or pathophysiological stimuli, these enzymes could be activated in a temporal and spatial sequence and ROS could be generated in different time periods and at different positions from various sources in a cell. ROS is probably produced by the activation of certain enzymes that could quickly respond to intra- or extracellular stimulation, such as NOX. Under continued pathological conditions, it is likely that ROS would mainly come from metabolic burden, such as OXPHOS and ER stress. On the antioxidant side, "Non-enzymatic Antioxidant Chain" comprises SOD, CAT, GCL, GPx, GSTs, Trxs, Prxs, NQO1, HO-1, SeP, MTs, and other undefined enzymes that may exhibit antioxidant activities, in combination with nonenzymatic antioxidants, constituting the first in vivo defense line against potential ROS insult. This defense chain could be reinforced by the consumption of naturally extracted antioxidants that are commercially available. On a higher level, the antioxidant enzymes constitute the "Antioxidant Enzyme Chain," a more efficient

defense line against potential ROS damage. These enzymatic and nonenzymatic antioxidants comprise the "Combined Antioxidant Chain," which sequentially and cooperatively regulate redox state. As the super directors, the redox-regulating transcription factors, such as Nrf2, PGC-1α, p53, NF-κB, and FoxO, form the "Transcription Factor Chain," which direct the battle between oxidants and antioxidants through transcriptionally regulating both ROS-generating enzymes and antioxidant enzymes. Moreover, there are additional mechanisms of redox regulation, including epigenetic and posttranslational regulation. The regulation of "Redox Chain" could be implemented in a temporal and spatial way. In all, more work is needed to clarify the role of each member in redox family and its regulatory mechanisms under normal and pathophysiological conditions.

Disturbance of "Redox Chain" results in either oxidative stress or blockage of ROS signaling transduction, leading to various disorders. In the context of obesity or enhanced adipocyte differentiation, redox state is altered as reflected by disorganized "Redox Chain" at different levels. However, we only observed changes or roles of single or several members of "Redox Chain" in adipocyte differentiation. Clarification of temporal and spatial changing pattern of "Redox Chain" in an integrated way will undoubtedly contribute to the understanding of the mechanisms underlying adipocyte differentiation and obesity.

3.30. ROS acts as a double-edged sword in the course of adipocyte differentiation

Based on the large amount of paradoxical results, it is difficult to draw a "clear" conclusion of the role of redox regulation in adipocyte differentiation. What we know is that adipocyte differentiation is definitely determined either directly by redox system or indirectly by redox-sensitive transcriptional, posttranslational, or epigenetic regulation. Compared with preadipocytes, mature adipocytes are under relatively more oxidizing state. In the physiological process of adipocyte differentiation, ROS is a definitely required and essential signal for the initiation and maintenance of adipogenic events. However, ROS-generating signals may be limited to a certain concentration and sources in different stages of adipogenic differentiation. We propose that in addition to the differential experimental backgrounds, including differences in experimental subjects and interventions, the paradoxical results from different laboratories may be attributed to the "narrow safety window" of concentration, time and compartment for the redox-regulated adipogenic differentiation. The difference of genetic or dietary background of the subjects, doses or periods of interventions or "time window" selected for detection may severely affect the outcomes. Concentration is a key determinant of the effect of ROS on adipocyte differentiation. Turker et al. reported that 1 and 10 μM H_2O_2 resulted in a marked decrease in adipocyte differentiation, while higher doses of H_2O_2 markedly increased differentiation. Both deficiency and over-expression of XOR have been shown to inhibit adipocyte differentiation. In the course of adipocyte differentiation, cells undertake dramatic transformation of cell fate, involving drastic changes of organelles and compartments. In differential stages of adipogenesis, redox state in different organelles and compartments may be differential. These data demonstrated that redox state required for adipocyte differentiation must be controlled in a fine-tuned way, including appropriate concentration, time, and space. The paradoxical results indicate the importance of delicate regulation of redox system in adipocyte differentiation. Due to the quick redox reaction and extremely

short half-life period, to date, the absolute amount of ROS could not be determined accurately in biological samples, and thus the ROS level we evaluated is a relative index. In different experiments, ROS level could not be compared. Thereof, over-expression or inhibition of a certain molecule (transcription factor, antioxidant enzyme, or ROS-generating enzyme) could not reflect the accurate level of ROS or redox state. Moreover, the adipogenic differentiation requires physiologically and endogenously generated ROS to create an oxidizing environment and to transduce molecular signals. The differentiation of adipocytes needs the accurate generation of ROS at the appropriate "time window" and "place." However, under pathological and stress conditions, ectopic and excessive generation of ROS may influence the redox environment and disturb adipogenesis. It is proposed that ROS acts as a double-edged sword in the course of adipocyte differentiation and thus in the pathogenesis of obesity and related metabolic disorders (**Figure 2**) [12].

3.31. Implications for intervention of obesity and associated redox-regulated disorders

During adipocyte differentiation, cells undertake dramatic and extensive morphological, physiological, and biochemical changes. These great changes render redox alteration the most

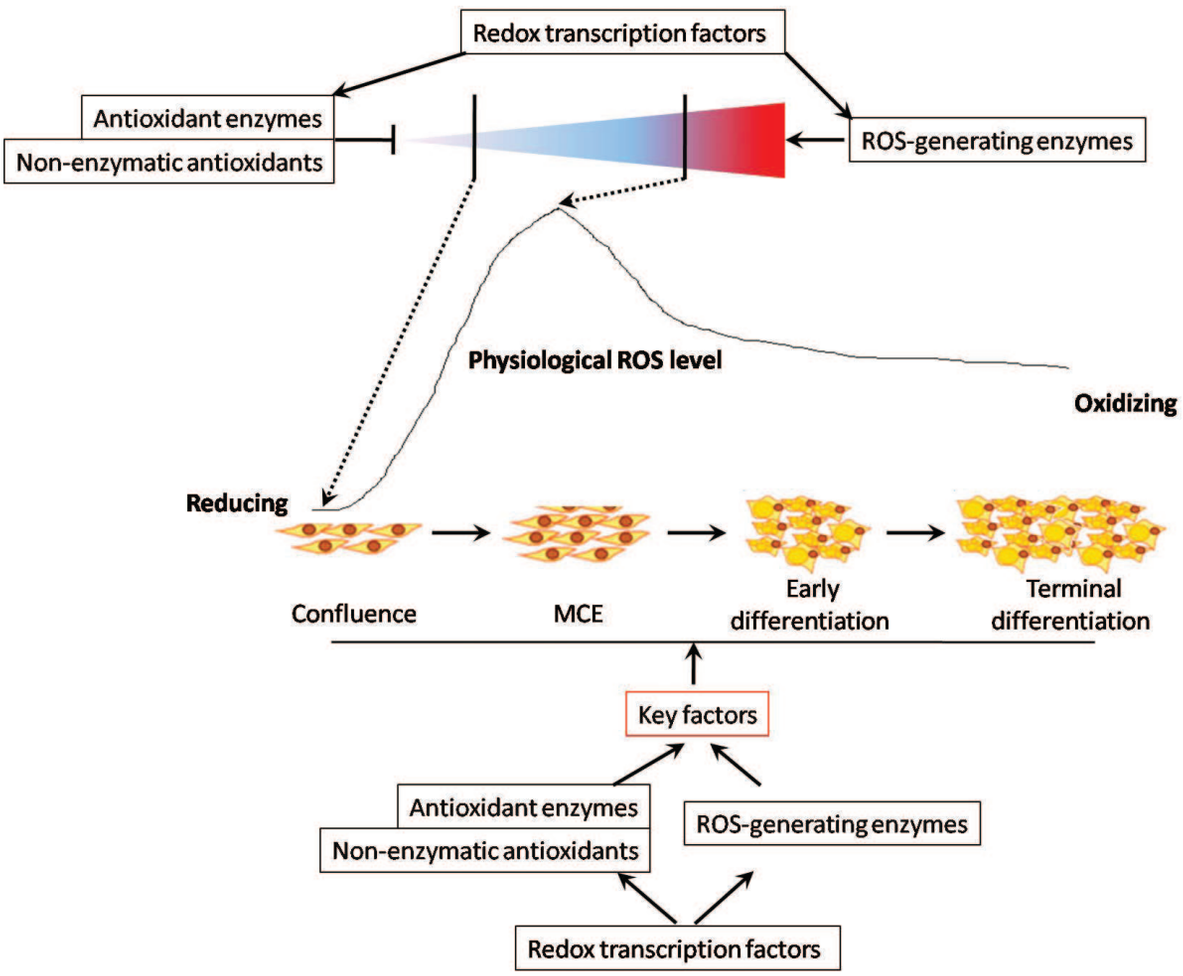

Figure 2. Role of redox system in the regulation of adipocyte differentiation.

probable "candidate" that could mediate those extensive reactions. The large amount and extensive distribution of members in redox family provide a general and microenvironment for thousands of reactions.

Adipocyte differentiation is a time-dependent process, which consists of different stages, including confluence, MCE, early differentiation, and late differentiation. In different stages of adipocyte differentiation, redox status is dramatically altered. It appears that redox system works as an "on-off switch" and orchestrates the transition from preadipocyte to adipocyte. During this process, redox regulation on the expression of key proteins for adipocyte differentiation may occur in diverse levels, including transcriptional, epigenetic, and posttranslational modulations (**Figure 3**). Indeed, the "tridimensional" redox regulatory mechanism is not limited in the process of adipocyte differentiation, but has general implications in a variety of biological processes.

A specific molecular mechanism would definitely play a role in a given background. However, one molecule or even one signaling pathway could not account for all the redox-sensitive events. Thereof, a more integrated view of redox biology should be highlighted. Considering the burst of various omics concept and technology, we suggest that redoxomics should be applied extensively and widely to help us understand the biological effects of redox alterations in a more integrated way. We need a stable and standardized "redox index" to evaluate the general redox status under a certain condition. Just like BMI, "redox index" represents a value to assess the overall situation of redox state in an organ or in a whole body. In our future work, we need to find the representative and easily obtained indicators and establish a simple equation to calculate "redox index." Moreover, adipose tissue-targeted redox interventions may be more attractive because disturbance of insulin signaling in other main organs, such as liver, could also result in insulin resistance and metabolic syndromes. Differential redox state in different organs may influence the overall metabolic end-points by using antioxidants. Our aim is to establish and maintain general and fine-tuned redox balance rather than to conduct simple prooxidant and antioxidant interventions. Individualized intervention according to their respective redox state should be emphasized to treat obesity and other redox-related metabolic disorders [13].

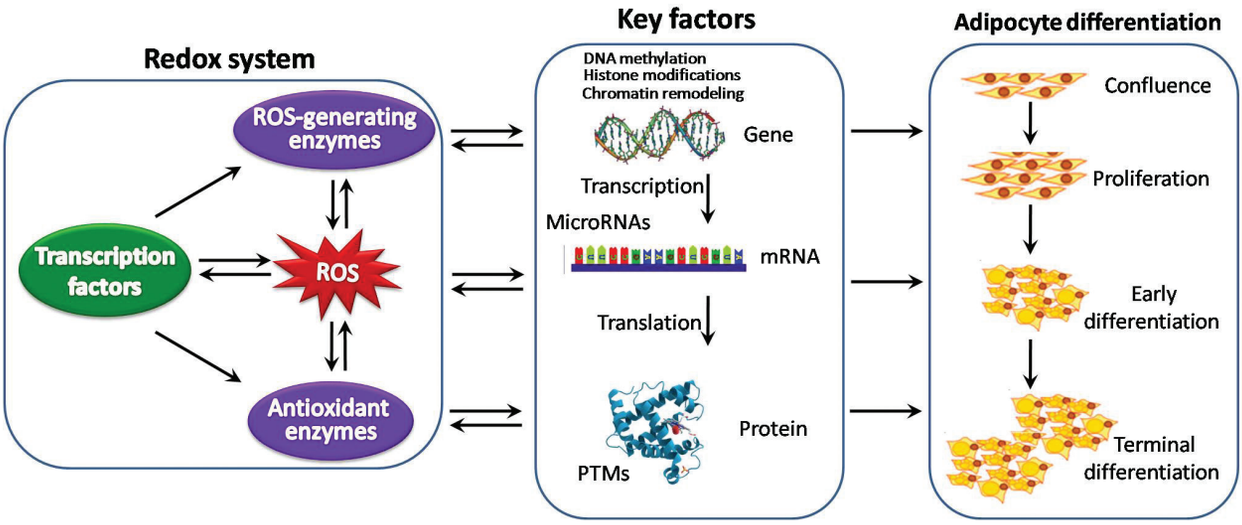

Figure 3. The "tridimensional" redox regulatory mechanism in the process of adipocyte differentiation.

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