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Review

Redox Mechanisms in Regulation of Adipocyte Differentiation: Beyond a General Stress Response

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Abstract: In this review, we summarize advances in our understanding of redox-sensitive mechanisms that regulate adipogenesis. Current evidence indicates that reactive oxygen species may act to promote both the initiation of adipocyte lineage commitment of precursor or stem cells, and the terminal differentiation of preadipocytes to mature adipose cells. These can involve redox regulation of pathways mediated by receptor tyrosine kinases, peroxisome proliferator-activated receptor γ (PPARγ), PPARγ coactivator 1α (PGC-1α), AMP-activated protein kinase (AMPK), and CCAAT/enhancer binding protein β (C/EBPβ). However, the precise roles of ROS in adipogenesis *in vivo* remain controversial. More studies are needed to delineate the roles of reactive oxygen species and redox signaling mechanisms, which could be either positive or negative, in the pathogenesis of obesity and related metabolic disorders.

Keywords: adipocyte; adipogenesis; differentiation; obesity; oxidative stress; reactive oxygen species; redox regulation

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1. Introduction

Adipogenesis is a process by which new adipocytes formed from mesenchymal stem cells or other precursor cells [1,2]. Expansion of the white adipose tissue results in development of obesity, which has significant contributions to hyperglycemia, hyperlipidemia, insulin resistance, chronic inflammation, type 2 diabetes and atherosclerosis [1,2]. Adipocyte differentiation and maturation is a complex developmental process involving a highly orchestrated program of gene expression, and understanding of the molecular mechanisms underlying adipogenesis is important for discovery of new targets to treat obesity-related disorders [3]. Excessive production of reactive oxygen species (ROS) induces oxidative stress in cells. This may lead to cellular damage caused by oxidative modification of lipids, proteins, and DNA [4]. However, it is becoming clear that a non-toxic level of ROS may be involved in transducing intracellular signals and thereby regulating fundamental cell behaviors such as proliferation, differentiation and survival [5–7]. Current evidence indicates that ROS may be involved in promoting both of initiation of adipocyte lineage commitment of precursor or stem cells, and the terminal differentiation of preadipocytes to mature fat cells. In this mini-review, we summarize advances in our understanding of redox-sensitive mechanisms that regulate adipogenesis.

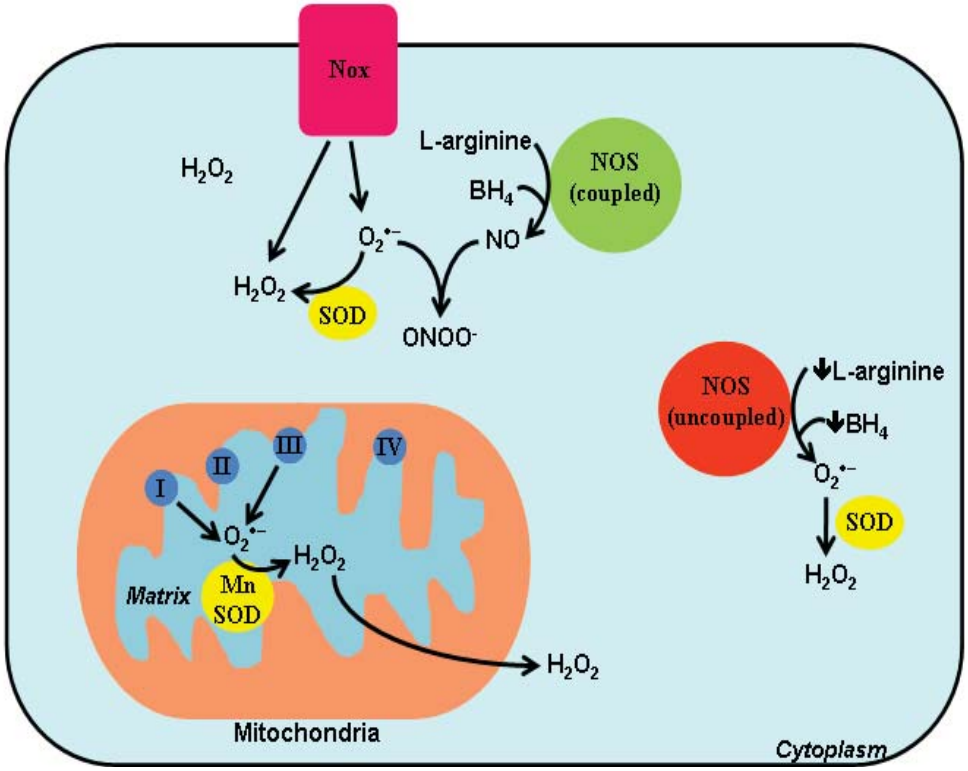
2. ROS and Redox-Dependent Signaling Mechanisms

ROS are oxygen-containing, short-lived molecules that are highly reactive. The most common examples include superoxide, hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), nitric oxide (NO) and peroxynitrite ($ONOO^-$). ROS are generally considered as unwanted by-products produced from aerobic metabolism, for instance the superoxide molecules during mitochondrial oxidative phosphorylation [8]. If ROS are not removed efficiently by endogenous antioxidant systems, such as superoxide dismutase (SOD), catalase and glutathione peroxidase, they may cause cellular oxidative stress and subsequent cellular damages. Oxidative stress is implicated in pathogenesis of many diseases including cardiovascular disease, diabetes and cancer [9,10]. On the other hand, emerging evidence suggests that ROS generated by cells at a physiological (non-toxic) level could act as transmitters in signaling pathways [5–7]. Some well-documented cellular signaling proteins that are sensitive to redox regulation include protein tyrosine phosphatases (PTP), certain serine/threonine and tyrosine kinases, and redox-responsive transcription factors (reviewed in [5,6,11,12]). The presence of redox-sensitive sulfhydryl groups in the key cysteine residues in these signaling proteins or their binding partners is essential for their responsiveness to ROS molecules. Oxidation and reduction of the cysteine sulfhydryl group may act as an "on-off" switch for changing structural configuration of the protein and thereby affecting the activity/affinity of the target. Some transcription factors may be activated by ROS, leading to enhanced target gene expressions in the presence of oxidants. In contrast, oxidation of the key cysteine residues in PTP may inactivate the enzyme, resulting in enhanced signaling events downstream of receptor or non-receptor tyrosine kinases.

ROS can be produced by various enzymes inside the cells (see Figure 1). NADPH oxidase is an important source of intracellular ROS implicated in redox signaling. NADPH oxidase is a multimeric enzyme, and seven isoforms of the catalytic subunit have been identified in mammalian cells, known as Nox1 to Nox5, Duox1 and Duox2. All isoforms have the catalytic domain that allows transport of

electron from cytosolic NADPH to generate superoxide, which is then converted to H_2O_2 by endogenous SOD [13]. Among these Nox isoforms, Nox4 is highly expressed in preadipocytes [14] and may be involved in modulating preadipocyte proliferation and/or differentiation [15,16]. Unlike other Nox proteins, Nox4 can generate ROS under basal conditions in the absence of exogenous stimuli [13]. Interestingly, Nox4 is the only isoform that primarily produces H_2O_2 instead of superoxide [13], and this property of Nox4 may be attributable to an extracellular domain called E-loop, which is longer than that of Nox1 or Nox2 [17]. Mutation of a highly conserved histidine in E-loop abolished the ability of Nox4 for spontaneous dismutation of superoxide to H_2O_2. It appears that H_2O_2 is the likely signal mediator for regulating cell proliferation, differentiation, migration because it is more cell permeable and has a relatively longer half-life in comparison to superoxide [18].

Figure 1. Sources of intracellular ROS generation. Among Nox isoforms (Nox1 to Nox5 and Duox1 and Duox 2), Nox4 is the only isoform that primarily generates hydrogen peroxide (H_2O_2) instead of superoxide ($O_2^{\bullet-}$). Superoxide is converted to H_2O_2 by endogenous superoxide dismutase (SOD). In mitochondria, superoxide is produced from complexes I and III and is then converted to H_2O_2 by manganese SOD (MnSOD). Nitric oxide synthase (NOS) catalyzes the formation of NO from L-arginine; however, NOS can be uncoupled under certain pathological conditions to produce superoxide when the availability of tetrahydrobiopterin (BH_4) or L-arginine is too low.



Other important sources of intracellular ROS generation include mitochondria and nitric oxide synthase (NOS). Mitochondria are the core machinery for energy production through oxidative phosphorylation [19]. Superoxide can be generated from one electron reduction of molecular oxygen in complex I and complex III and is then converted to H_2O_2 by the mitochondrial manganese SOD [8].

There are other enzymatic sources of ROS generation within mitochondria, such as α-ketoglutarate dehydrogenase [20] located in the matrix, and monoamine oxidase localized at the outer membrane [8]. The biological roles of mitochondria-mediated redox signaling have been extensively reviewed by others [21]. NOS catalyzes the formation of NO from L-arginine via two successive oxygenation steps involving electron transfer process between the C-terminal flavin-containing reductase domain and N-terminal heme-containing oxygenase domain [22]. Under certain pathological conditions, the enzymatic activity of NOS can be uncoupled to produce superoxide rather than NO. For example, when the concentration of NOS cofactor tetrahydrobiopterin or substrate L-arginine is too low [22]. This oxidation of tetrahydrobiopterin facilitates superoxide generation from endothelial cells [23]. The oxidized form of tetrahydrobiopterin is unable to donate electron to synthesize NO, while $ONOO^-$ is formed from concomitant generation of superoxide and NO by uncoupled NOS.

3. Molecular Mechanisms Governing Adipocyte Differentiation

Preadipocytes are thought to be derived from mesenchymal stem cells, which are capable of differentiating into multiple lineages including adipocytes, osteoblasts, chondrocytes, and myoblasts [24,25]. Adipogenic process occurs via two components: commitment of mesenchymal stem cells toward preadipocyte fate, and terminal differentiation of preadipocytes into mature adipocytes [25]. Several signaling pathways have been discovered that are sequentially involved in regulating adipogenesis [26]. There is evidence that bone morphogenetic proteins (BMP) BMP2 and BMP4 may induce stem cell commitment to adipocytes [27,28]. In contrast, the canonical Wnt signaling has been shown to inhibit adipogenesis by shifting mesenchymal stem cell fate toward the osteoblast lineage [29,30]. Cell cycle arrest of preadipocytes induced by contact inhibition is essential for the initiation of differentiation to adipocytes [31]. These cells in growth arrest state then undergo a process known as mitotic clonal expansion, in which cells re-enter the cell cycle and repeat several rounds of cell division stimulated by adipogenic stimuli such as cAMP elevating agents, glucocorticoids and insulin [25,32]. These processes coincide with the early phase of adipocyte differentiation [1]. Among the transcription factors activated in the early phase of adipocyte differentiation, CCAAT/enhancer binding protein β (C/EBPβ) and C/EBPδ are the major effectors [33]. C/EBPβ activity is regulated by multiple mechanisms including transcription, phosphorylation and acetylation [25]. C/EBPδ expression level shows a surging response in the early phase and almost disappears in the late phase [1]. cAMP upregulates C/EBPβ via cAMP response element-binding protein (CREB) and Krüppel-like factor 4 (KLF4) [34,35]. Another mediator of C/EBPβ transcription is Krox20, which is rapidly induced by mitogens and, together with KLF4, cooperatively transactivates the C/EBPβ promoter in 3T3-L1 [34,36]. Signal transducer and activator of transcription 3 (STAT3), which is downstream of the Janus kinase 2 (JAK2), regulates C/EBPβ transcription by binding to the distal region of C/EBPβ promoter [37]. Moreover, C/EBPβ needs to be sequentially phosphorylated by mitogen-activated protein kinases (MAPK) and glycogen synthase kinase (GSK)-3β to acquire DNA binding activity [38]. Glucocorticoid, an essential adipogenic agent, enhances C/EBPδ expression [39] and promotes C/EBPβ acetylation, leading to potentiated C/EBP-dependent adipogenic differentiation [40]. Terminal differentiation of adipocytes is primarily regulated by transcription factors C/EBPα and peroxisome proliferator-activated receptor (PPAR)γ,

which program gene expressions required for mature adipocytes [33,41]. C/EBPβ and C/EBPδ, along with glucocorticoid receptor, STAT5, and retinoid X receptor (RXR), stimulate expression of PPARγ and C/EBPα [25,33,42]. Subsequently, PPARγ and C/EBPα cooperate to induce expression of adipocyte specific genes such as FABP4, GLUT4, adiponectin, PEPCK, CD36, LPL, AGPAT2, PLIN1, and LEP [43–46].

PPARγ is the master regulator of terminal differentiation and gene expression during adipogenesis, and many adipogenic signaling pathways target PPARγ activity. Insulin induces phosphorylation of Akt, which then activates mammalian target of rapamycin complex 1 (mTORC1) via inhibition of TSC1/2, leading to activation of SREBP1c, an adipogenic transcription factor which regulates fatty acid synthase, lipoprotein lipase and PPARγ expression [47–51]. Akt also enhances PPARγ expression via nuclear exclusion of Foxo1, which inhibits PPARγ activity [52]. In addition, KLF5, which is upregulated by C/EBPβ and C/EBPδ, activates PPARγ2 promoter and induces 3T3-L1 adipogenic differentiation without hormonal stimulation [53]. Similarly, KLF15 also promotes PPARγ expression and lipid accumulation [54]. In contrast, KLF2 functions as a negative regulator of adipocyte differentiation by inhibiting PPARγ expression [55]. Moreover, sirtuin proteins are also involved in modulating lipid and glucose metabolism by suppressing PPARγ activity [56]. For example, Sirt1 may repress PPARγ function by binding to its cofactors [57], while Sirt2 may repress PPARγ transcriptional activity by activating Foxo1 [52,58]. PPARγ coactivator 1α (PGC-1α) is a transcriptional regulator, which may act as a coactivator of PPARγ. It has been well documented that PGC-1α has critical roles in modulating the expression of genes involved in energy metabolism, including mitochondrial biogenesis, glucose uptake, gluconeogenesis, fatty acid oxidation, and adaptive thermogenesis [59–61]. Several lines of evidence suggest that activation of PGC-1α favors formation of brown adipose and maintains the specific thermogenic function of brown fat cells [62,63].

4. Emerging Evidence of Redox-Dependent Regulation of Adipogenesis

Several lines of studies have suggested that intracellular ROS derived from NADPH oxidase, mitochondria, and NOS may have a significant role in modulating adipocyte differentiation (summarized in Table 1). There is evidence that NADPH oxidase is a major source of ROS in preadipocytes [14,64]. We and others have shown that elevated expression of NADPH oxidase subunits can be observed in adipose tissues in rodent models of obesity and in humans with extreme insulin resistance [64–66]. During differentiation of 3T3-L1 preadipocytes, there was a marked increase in ROS production, which was blocked by inhibitors of NADPH oxidase [64]. In consistent with these data, our previous study demonstrated that in human adipose-derived stem cells, agonists-induced adipogenic differentiation was accompanied by increased ROS generation, while scavenging ROS production inhibited the induced adipogenesis process [15]. Moreover, we found that inhibition of NADPH oxidases suppressed adipogenesis in these cells. Of the different Nox isoforms of NADPH oxidase, studies have focused on Nox4, as Nox4 is highly expressed in preadipocytes [9,14]. Kanda *et al.* found that in mesenchymal stem cells, Nox4-produced intracellular ROS enhanced adipogenic differentiation [15]. Similarly, Schröder and coworkers showed that in 3T3-L1 cells, inhibiting Nox4 expression by gene silencing blocked insulin-induced terminal differentiation to adipocytes [16]. These results suggest that Nox4 may have a positive role in promoting the

adipogenesis process, probably by facilitating insulin signaling [67]. Despite these *in vitro* data, however, the precise role of Nox4 in adipogenesis needs to be confirmed in other cell types, and its role *in vivo* remains elusive. For example, it was shown that Nox4 deficiency accelerated development of obesity and insulin-resistance in mice [68]. Moreover, the involvement of other Nox isoforms in adipogenesis is currently unclear.

Table 1. Intracellular sources of ROS implicated in modulating adipocyte differentiation

Source of ROS	Experimental models	Primary findings	References
NADPH oxidase	3T3-L1 adipocytes	Increased ROS production in accumulated fat contributes to metabolic syndrome.	[64]
NADPH oxidase	3T3-L1 adipocytes, human preadipocytes	Nox4 acts as a switch from insulin-induced proliferation to differentiation by controlling MKP-1 expression, which limits ERK1/2 signaling.	[16]
NADPH oxidase	Mouse MSCs	Increase in the intracellular ROS level via Nox4 mediates adipocyte differentiation through CREB in MSC.	[15]
Mitochondria	3T3-L1 adipocytes	Increase in mitochondrial ROS production caused by inhibition of the electron transport chain (complex I and V) prevented preadipocyte proliferation.	[69]
Mitochondria	Human MSCs	Mitochondrial metabolism and ROS generation are not simply a consequence of differentiation but are a causal factor in promoting adipocyte differentiation.	[70]
NOS	Rat preadipocytes	NO is involved in the positive modulation of preadipocyte differentiation and eNOS rather than iNOS may be the major isoform involved in modulating adipogenesis.	[71]

ROS: reactive oxygen species; MSC: mesenchymal stem cells; NO: nitric oxide; eNOS: endothelial nitric oxide synthase; iNOS: inducible nitric oxide synthase.

The role of mitochondria in adipocyte differentiation is not well understood, but several studies have suggested that ROS produced from dysfunctional mitochondria are associated with altered adipocyte function in diseases such as the metabolic syndrome, diabetes and obesity. Carriere *et al.* studied the role of mitochondrial ROS in regulation of preadipocyte proliferation in 3T3-L1 cells, and demonstrated that an increase in mitochondrial ROS production caused by inhibition of the electron transport chain (complex I and V) prevented preadipocyte proliferation [69]. However, it was observed that in the early phase of adipocyte differentiation of human mesenchymal stem cells, there was an increase in mitochondrial metabolism and ROS generation. Moreover, the authors demonstrated that ROS production from mitochondrial complex III was required for activation of the adipogenic transcriptional cascade via upregulation of C/EBPα and PPARγ [70]. In agreement with these results in human mesenchymal stem cells, we found that inhibiting mitochondrial ROS production with rotenone partially suppressed adipogenic differentiation of human adipose-derived stem cells [71].

Hence, the role of mitochondria-derived ROS in regulating adipogenesis is complex, and appears to be cell type specific. Both endothelial NOS (eNOS) and inducible NOS (iNOS) can be expressed in (pre)adipocytes [72]. With the identification of eNOS and iNOS in adipose cells, there is increasing evidence suggesting that NO (which is also called a reactive nitrogen species or RNS molecule) may have a pivotal regulatory role in adipocyte physiology. For example, it was demonstrated that NO promoted adipogenic differentiation of rat preadipocytes [71]. In vitro differentiation of preadipocytes was accompanied by an increase in iNOS expression, while insulin and angiotensin II increased NO production by preadipocytes [73]. It is likely that NO promotes adipogenesis through activation of the cGMP-PKG pathway [74]. Different clinical studies further demonstrated that the expression levels of NOS and NO production were augmented in the adipose tissue from obese subjects, suggesting that NO might have a role as a modulator of adipogenesis in obesity [73–77]. Nevertheless, the precise role of NOS in adipogenesis needs to be confirmed by further studies.

Regardless of the specific sources of intracellular ROS production, the close relationship between ROS and adipogenesis has been confirmed by a number of recent studies [78–85]. Different approaches were used in these studies, including pharmacological treatment with antioxidant agents, genetic manipulation of gene expression, and direct measurement of intracellular redox status. Observations form several studies also showed that differentiated adipocytes are metabolically distinct from preadipocytes for adipocytes produce much higher basal levels of intracellular ROS than preadipocytes [16,86,87]. Overall, these results strongly suggest that a more oxidized intracellular environment favors differentiation of progenitor or stem cells into mature adipocytes. Recently, we observed that in human adipose-derived stem cells, overexpression of Nox4 and exogenous application of H_2O_2 boosted terminal differentiation into mature adipocytes, further supporting a positive regulatory role of ROS in adipogenesis [88]. Moreover, these *in vitro* data are complemented by the finding that systemic administration of the superoxide scavenging agent tempol in mice prevented the development of obesity [89].

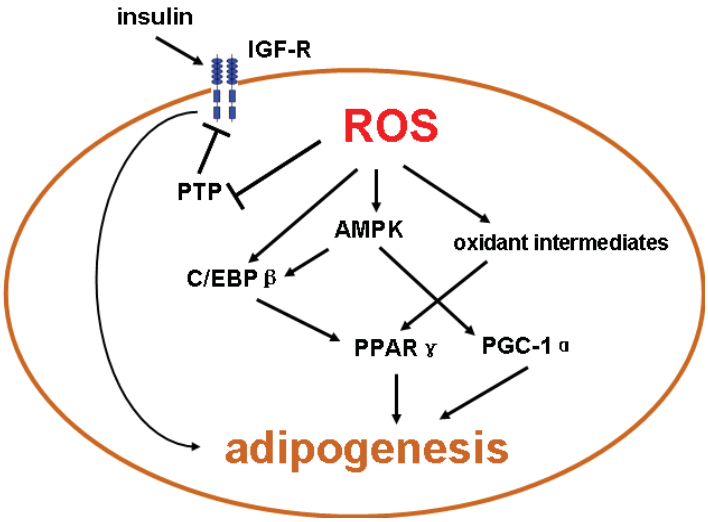
Interestingly, an adipocyte phenotype can be induced by various stimuli in non-adipogenic cells via trans-differentiation [90–93]. For example, treatment with interleukin-17 promoted trans-differentiation of a mouse myoblast cell line into adipocytes [90]. Likewise, it was shown that inhibition of the connexin function with glycyrrhetinic acid in skeletal muscle cells induced a phenotype change into the adipocyte lineage [92]. Overall, these studies suggest that adipocyte trans-differentiation from non-adipogenic cell types is likely to be mediated by a set of transcriptional regulators that are similar to those involved in conventional adipogenesis, such as PPARγ and C/EBP proteins [90–93]. However, whether cell trans-differentiation into adipocytes is also regulated by redox mechanisms remains to be clarified.

5. Redox-Sensitive Mechanisms Related to Adipogenesis

How ROS regulate adipogenesis is not completely understood. Different lines of studies have provided some insights into the biological mechanisms underlying redox regulation of adipogenesis (Figure 2). Insulin-like growth factor-1 (IGF-1), an anabolic hormone, has been shown to promote the differentiation of preadipocyte to adipocytes [94]. IGF receptor mediated signaling is responsible for IGF-1 or insulin-induced adipogenesis in 3T3-L1 cells [94]. The IGF receptor is a tyrosine kinase that

activates a series of signaling pathways, including the MAPK ERK1/2. It was demonstrated that exposure of 3T3-L1 preadipocytes to an insulin-containing adipogenic cocktail activated ERK1/2, while treatment with ERK pathway inhibitors U0126 or PD98059 significantly attenuated expression of C/EBPα and PPARγ [95]. More than three decades ago, people found that H_2O_2 could mimic the metabolic effects of insulin in adipocytes [96,97]. Accumulating evidence now suggests that ROS such as H_2O_2 may act as signaling messengers to facilitate biological functions of insulin and IGF-1, primarily by inhibiting endogenous PTP activities [98–100]. Likewise, the ERK pathway downstream of receptor tyrosine kinases has been shown to be sensitive to redox regulation in different cells [7]. Indeed we recently demonstrated that Nox4 overexpression in human endothelial cells augmented basal phosphorylation of ERK1/2 [101]. Moreover, ROS have been shown to regulate ERK activation in adipose-derived stem cells [102], which are thought to be precursors of preadipocytes [103]. Current evidence supports that ERK pathway has an important role in the initial lineage commitment of preadipocytes during the differentiation process, but may also have an inhibitory effect on adipocyte maturation [104]. In addition to the ERK pathway, ROS may have positive regulatory actions on other signaling components downstream of the IGF receptor tyrosine kinase that are important to adipogenesis.

Figure 2. Potential redox-sensitive pathways involved in regulation of adipogenesis. C/EBPβ, CCAAT/enhancer binding protein β; IGF-R, insulin-like growth factor receptor; PTP, protein tyrosine phosphatases; PGC-1α, PPARγ coactivator 1α; AMPK, AMP-activated protein kinase.



Another adipogenesis-related signaling molecule that might be regulated by redox mechanisms is PPARγ, which is a master regulator of adipogenesis [1,41]. In an epithelial cell line, treatment with an oxidant compound tert-butylhydroperoxide increased the transcriptional activity of PPARγ [105]. In addition, many oxidatively modified biomolecules have been shown to activate PPARγ. For example, UV irradiation induced PPARγ activation in human epithelial like cells, which was mediated by oxidized glycerophosphocholine species with intrinsic PPARγ ligand activity [105]. Similarly, Nagy *et al.* demonstrated that an oxidative product of linoleic acid, hydroxyoctadecadienoic acid, acted as endogenous PPARγ activators and mediated oxidized low density lipoprotein-induced gene

expressions in macrophages [106]. The mechanisms of redox-dependent regulation of PPARγ activity remain largely unknown. There is evidence that the Cys-285 located in the LBD domain of PPARγ is redox-sensitive, and the electrophilic nitro-fatty acids can act as partial agonists of PPARγ through covalent binding to Cys-285 [107]. Nitro-fatty acids can be formed by nitration of unsaturated fatty acids under an oxidizing and nitrating condition that could occur during inflammation. So far, however, there is no evidence that PPARγ can be activated by ROS-mediated Cys-285 oxidation, suggesting that oxidative stress-induced PPARγ activation requires an oxidative intermediate. Moreover, although an inhibition of glutathione synthesis has been shown to increase PPARγ expression in 3T3-L1 cells, whether PPARγ can be activated by oxidative stimuli in (pre)adipocytes, and its role in adipogenesis, would require further investigations.

PGC-1α may also be under redox regulation. In vitro, oxidative stress could induce PGC-1α expression, which was required for the induction of many antioxidant enzymes including glutathione peroxidase and mitochondrial superoxide dismutase (SOD2), indicating that PGC-1α might have an important role in regulating intracellular redox homeostasis [108]. Moreover, PGC-1α has been shown to be a direct substrate of AMP-activated protein kinase (AMPK), and phosphorylation of PGC-1α by AMPK enhanced its transcriptional activity in skeletal muscle cells [109]. Since accumulating evidence has suggested that the functions of AMPK may be tightly regulated by redox status [110], it is plausible that ROS may participate in modulating PGC-1α function, at least indirectly. Supporting these *in vitro* findings, Kang *et al.* carried out an *in vivo* study in rats and demonstrated that endurance exercise increased muscle xanthine oxidase activity and ROS generation. Exercise also upregulated PGC-1α expression, an response that was attenuated by inhibition of xanthine oxidase, suggesting that exercise-activated PGC-1α signaling pathways in skeletal muscle were redox sensitive [111]. Like PPARγ, the nature of redox regulation of PGC-1α signaling and its effects on adipogenic differentiation in precursor or tem cells remain to be elucidated.

Moreover, redox status may affect the activity of C/EBPβ. It was shown that an oxidative condition further increased the DNA binding of doubly phosphorylated, but not unphosphorylated, C/EBPβ [112]. ROS induced disulfide bond formation between cysteine residues and dimerization of C/EBPβ, leading to further increases in DNA binding activity. This mechanism was supported by the finding that mutation of Cys296 and Cys143 diminished phosphorylation-, oxidation-, and dimerization-dependent DNA binding activity of C/EBPβ [112]. Moreover, it was shown that in 3T3-L1 cells, H_2O_2 accelerated hormone-induced adipogenic differentiation with increased expression of PPARγ [113]. H_2O_2 also enhanced the mitotic clonal expansion process by promoting cell cycle progression from S to G2/M phase, while treatment with antioxidants resulted in S phase arrest. Oxidant treatment in 3T3-L1 cells resulted in the early appearance of C/EBPβ puncta, which is the characteristic morphological change for enhanced C/EBPβ DNA binding, whereas antioxidant treatment rapidly dispersed the C/EBPβ puncta [113]. All of these results suggest that oxidative stress may facilitate adipogenesis by modulating C/EBPβ-mediated gene expression program.

6. Conclusion Remarks

Redox mechanisms have critical roles in regulating cellular metabolism, and oxidative stress is an important element in metabolic disorders [114–116]. Human studies have revealed that there is a

correlation between fat accumulation and systemic oxidative stress in obese people [115–118]. However, our understanding of the reciprocal relationships between obesity and ROS production remains incomplete. Studies have suggested that ROS may be involved in promoting both of the early differentiation and the subsequent maturation of adipose cells [15,70,78–85,88]. However, the role of ROS in adipogenesis *in vivo* is still controversial, and needs more investigation [68,119]. In adults, obesity is mainly caused by hypertrophy of adipocytes with increased lipid accumulation, while de novo adipocyte formation from stem cells might be responsible for continuous turnover of fat cells in the body [2]. Interestingly, emerging evidence has indicated that in hypertrophic obesity, the number of preadipocytes in the subcutaneous adipose tissue capable of undergoing adipogenic differentiation to mature adipocytes is paradoxically reduced, and it is anticipated that restoring the impaired preadipocyte differentiation in the subcutaneous adipose tissue may be a new approach to prevention of ectopic lipid accumulation and resultant insulin resistance [2]. Adding to this complexity is the multi-facet actions of ROS in cell biology. It is likely that ROS may produce disparate effects on adipogenesis depending on the time, intracellular location and intensity of ROS generation. Therefore, we suggest that more carefully designed *in vivo* studies are needed to precisely define the roles that ROS and redox signaling mechanisms are playing, which could be either positive or negative, in obesity and related metabolic disorders.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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