New evaluations of redox regulating system in adipose tissue of obesity

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Abstract

During the past several decades, the incidence of obesity has significantly increased worldwide. Enormous efforts have been devoted to understanding the molecular mechanisms underlying obesity and its related metabolic disorders such as type 2 diabetes, cardiovascular disease, atherosclerosis, and hypertension. It is now well-established that altered adipocyte metabolism in obese patients is closely associated with the induction of various metabolic stresses including hyperglycemia, hyperlipidemia, hyperinsulinemia, and chronic inflammation. However, the cellular factor(s) which sense metabolic changes and/or initiate the pathological progression of obesity-induced metabolic disorders remain to be elucidated. In this review, we will discuss the possible roles of cellular NADP⁺/NADPH, which function as redox potential regulators, in the induction of obesity-associated oxidative stress, chronic inflammation, and insulin resistance and suggest G6PD, a NADPH-generating enzyme, as a novel target for treating metabolic disorders.

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The marked increase in the prevalence of obesity is recognized as one of the most serious public health issues in westernized countries [1]. Obesity causes significant increases in mortality due to metabolic disorders including type 2 diabetes, cardiovascular diseases, hyperlipidemia, and hypertension [2]. Much effort has been made toward understanding the molecular mechanisms underlying obesity-induced metabolic disorders and identifying putative targets for therapeutic intervention. In this review, we will focus on oxidative stress and chronic inflammation in adipose tissues that have recently been recognized as key mediators for the pathogenesis of metabolic disorders, and suggest a novel target to overcome obesity and related metabolic disorders.

1. Oxidative stress in fat cells

Recent studies have revealed that obesity is associated with cellular oxidative stress resulting from imbalance between reactive oxygen species (ROS) generation and scavenging. Oxidative stress affects several biological processes, such as cell cycle, metabolism, and gene expression, resulting in various progressive diseases such as cancer, neurodegenerative diseases, autoimmune diseases, and chronic inflammation [3–6]. Furthermore, it has recently been shown that increased oxidative stress in the adipose tissues of obese.
subjects is closely linked with enhanced inflammatory signals, adipocytokine dysregulation, and insulin resistance [7,8]. However, two key questions remain to be answered. (i) Which cellular factors increase oxidative stress in the adipose tissues of obese subjects? (ii) Which cellular factors sense and respond to oxidative stress in adipose tissues?

It has been proposed that hyperglycemia, mitochondrial dysfunction, and dysregulation of redox-regulating enzymes are involved in the production of cellular ROS in obesity (Fig. 1). For example, hyperglycemia promotes superoxide generation through the polyol pathway, hexosamine pathway, PKC pathway, and advanced glycation end-product (AGE) production, resulting in oxidative stress [9]. Mitochondrial dysfunction has been the focus of attention in deciphering the pathogenic mechanism of several oxidative stress-related disorders including vascular complications of diabetes, neurodegenerative diseases, and cell senescence [10–14]. However, it is yet unknown whether mitochondrial dysfunction in adipose tissues plays a role in the onset of obesity-induced metabolic disorders.

Another major source of cellular ROS is pro-oxidative enzymes such as nitric oxide synthase (NOS) and NADPH oxidases. These enzymes were shown to be significantly increased in the adipose tissues of obese subjects and appear to mediate obesity-induced insulin resistance [7,15]. Nitric oxide (NO) produced by iNOS is a key messenger molecule in various cellular processes including vasodilation, apoptosis, and energy metabolism [16–19]. Increased NO levels in obese subjects induce insulin resistance by disrupting the signaling pathways via IR, IRS1, and Akt in skeletal muscles [20–22] and cause β-cell apoptosis [23]. In addition, targeted disruption of iNOS in mice protects against diet-induced obesity and augments insulin sensitivity [15], suggesting that chronic induction of iNOS in obese subjects contributes to obesity-induced insulin resistance.

Superoxide producing-NADPH oxidases are also highly increased in adipose tissues of obese mice such as db/db and KKAy mice and induce oxidative stress and dysregulation of adipocytokines [7]. Because both iNOS and NADPH-oxidases utilize NADPH as a cofactor, it is likely that the demand for NADPH would be increased in obese adipose tissues. In somewhat paradox, NADPH is also essential for antioxidative processes such as the reduction of oxidized glutathione (GSSG) to its reduced form (GSH) by glutathione reductase (GR), which can scavenge ROS. The intracellular NADP+/NADPH ratio thus appears to be critical in determining the intracellular redox status, and its disruption may be involved in the generation of obesity-induced oxidative stress.

2. Obesity and systemic inflammation in fat cells

Adipose tissue is well-known as an endocrine tissue, which regulates whole body energy homeostasis by secreting various cytokines, also called ‘adipocytokines’ [24]. The expression and secretion of adipocytokines are altered in obesity, insulin resistance, atherosclerosis, cardiovascular diseases, and other metabolic disorders. Tumor necrosis factor α (TNFα), was the first adipocytokine suggested to be a molecular link between obesity and inflammation since its levels are increased in the adipose tissues of obese subjects and its increase impairs insulin sensitivity [25]. Several adipocytokines including TNFα, interleukin 6 (IL6), monocyte-chemoattractant protein 1 (MCP1), and resistin, are also expressed in macrophages and provoke insulin resistance and inflammatory signals (Fig. 1). Furthermore, adipose tissues in obese patients express abnormally high levels of inflammatory cytokines and recruit more macrophages, suggesting that cross-talks between adipocytes and macrophages in obese adipose
tissues might result in chronic inflammation and insulin resistance [26–28].

Elevated levels of circulating free fatty acids (FFAs) resulting from dysregulated lipid metabolism are also capable of promoting chronic inflammation and insulin resistance (Fig. 1). FFAs activate the PKC or NFκB pathways in liver, muscle, pancreatic β-cells, and the central nervous system even though the exact molecular mechanisms of such activation are not yet understood [29]. Although numerous studies have demonstrated that obese adipose tissues are in a state of chronic inflammation, it is unclear how macrophages are recruited to obese adipose tissues to trigger inflammatory signals.

3. Cellular redox changes in obesity and metabolic disorders

Fluctuations in NAD+/NADH or NADP+/NADPH ratios would affect crucial cellular metabolic processes because they are used as cofactors in many key metabolic pathways including glycolysis, oxidative respiration, reductive biosynthesis of lipids, and maintenance of redox balance. Cellular NADP+ is reduced to NADPH by several enzymes including the cytosolic glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), malic enzyme (ME), isocitrate dehydrogenase (IDPc), and the ER-residing hexose-6-phosphate dehydrogenase (H6PD). Interestingly, recent studies suggest that abnormal activation of G6PD and IDPc, resulting in increase of cellular NADPH, provokes lipid dysregulation and other pathological conditions observed in obesity [30,31]. For instance, IDPc transgenic mice exhibited obesity, hyperlipidemia, and fatty liver while glucose sensitivity was improved compared to wild type mice [30]. In addition, we recently demonstrated that G6PD expression is greatly increased in the adipose tissues of several obese and diabetic animal models, and that its abnormal increase causes dysregulation of lipid metabolism and adipocytokine expression, resulting in insulin resistance [31]. Furthermore, G6PD overexpression stimulates oxidative stress and inflammatory signals in adipocytes (Fig. 2A), which leads to increased recruitment and infiltration of macrophages into adipose tissues (Fig. 2B) [32].

The fact that overexpression of G6PD in adipocytes appears to be associated with obesity, insulin resistance, oxidative stress, and inflammation, makes it an attractive target for tackling obesity and related metabolic disorders. Even though further studies, including in vivo experiments using animal models, are required to obtain a better understanding of the roles of G6PD and other NADPH-producing enzymes, the evidence produced so far indicate that they could act as a ‘trigger’ during the onset of obesity-induced metabolic disorders. However, several critical questions still remain to be addressed, such as, whether the actual level of NADPH or change in NADP+/NADPH ratio is crucial for metabolic changes.

Interestingly, several enzymes which are regulated by cellular NADPH levels have recently emerged as important factors in the pathogenesis of obesity-related metabolic disorders (Fig. 3). Among them are NADPH

![Fig. 2. G6PD overexpression in adipocytes increases inflammatory gene expressions and THP-1 monocyte recruitment. (A) mRNA levels of inflammatory genes in G6PD overexpressing adipocytes. The 3T3-L1 adipocytes were infected with mock- or G6PD-adenovirus. Total RNA was isolated and analyzed by quantitative-real time-PCR (Q-PCR) for TNFα, IL6, MCP-1, chemokine receptor 2 (CCR2), Cox2, and Resistin. Results are represented as mean ± S.E. of three independent experiments performed in duplicates. * p < 0.05 vs. Adeno-Mock; ** p < 0.01 vs. Adeno-Mock by t-test. (B) G6PD overexpressing adipocytes exhibit increased association with monocytes, resulting in systemic inflammation. 3T3-L1 adipocytes infected with mock- or G6PD-adenovirus were cocultured with THP-1 monocytes for 2 days. Nonadherent monocytes were removed by washing with PBS and the attached monocytes to the adipocytes were immunostained with anti-CD68 antibody. Nucleus was stained with DAPI.](image-url)
oxidases and iNOS, which are involved in pro-oxidative pathways, and 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which converts inactive glucocorticoids into their active forms in target tissues. In adipose tissues, 11β-HSD1 locally increases cortisol levels, causing insulin resistance and visceral obesity [33–35]. Interestingly, it has been reported that H6PD and G6PD supplies the NADPH required for 11β-HSD1 activity [36,37], indicating a possible mechanism by which anomalous activation of NADPH producing enzymes might induce insulin resistance and other metabolic disorders.

In addition, NAD cofactors have made their appearance onto the transcriptional regulation scene. The recent discovery of three factors, Sirt1, Clock, and CtBP, whose activities are regulated by NAD(P)H/NAD(P)+ ratios, revealed the role of NAD cofactors in gene expression regulation, and suggest another possible mechanism by which cellular redox imbalance could cause abnormal metabolism [38–40]. For example, Sirt1 is an NAD+-dependent protein deacetylase that has been implicated in aging, cell differentiation, and cell survival (Fig. 3). More importantly, Sirt1 regulates lipid and glucose metabolism by sensing changes in intracellular energy levels and initiating the fasting response. Sirt1 increases lipolysis and fat release from fat cells by repressing PPARγ [41]. In addition, Sirt1 enhances insulin secretion from pancreatic β cells by repressing the expression of UCP2 [42,43]. Moreover, PGC-1α and FOXO1 are directly deacetylated and activated by Sirt1 in liver, resulting in the stimulation of gluconeogenesis [44,45]. Since FOXO1 is a downstream molecule of the insulin signaling pathway, it is possible that aberrant regulation of FOXO1 by Sirt1 could contribute to insulin resistance in diabetic patients. CtBP, initially discovered as a factor that repress the transcriptional activity of the adenovirus E1A protein [46], and Clock, an essential key transcription factor involved in mammalian circadian rhythm [47], are also regulated by intracellular levels of NAD cofactors [39,40,48]. The activities of Sirt1 and CtBP are regulated specifically by NAD+/NADH levels, while the DNA binding ability of Clock is affected by both NAD+/NADH and NADP+/NADPH ratios [38,40,48]. Recent studies have shown that Clock mutant mice develop obesity and metabolic syndromes, suggesting that circadian rhythm could be linked with energy homeostasis [49]. These findings suggest that the aberrant expression of several genes implicated in metabolic disorders may be mediated by the transcriptional regulatory function of NAD cofactors (Fig. 3).

A wide range of studies have suggested a very interesting involvement of cellular redox-regulating system in the pathogenesis of obesity-induced metabolic disorders. It seems likely that the various members of this system, ranging from NADPH producing enzymes to molecules whose activities are modulated by cellular NAD(P)H/NAD(P)+ levels, act as sensors and effectors during obesity. Therefore, future research into this field may pave the way for the development of a new therapeutic method against one of our most widespread foes, obesity and metabolic disorders.

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