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PERSPECTIVE

Reductive stress—a common metabolic feature of obesity and cancer

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Abstract Reductive stress, characterized by rising level of NADH (nicotinamide adenine dinucleotide) for a status of NADH/NAD^+ ratio elevation, has been reported in obesity and cancer. However, the mechanism and significance of reductive stress remain to be established in obesity. This perspective is prepared to address the issue with new insights published recently. NADH is used in production of NADPH, glutathione, ATP and heat in the classical biochemistry. In obesity, elevation of NADH/NAD^+ ratio, likely from overproduction due to substrate overloading, has been found in the liver for insulin resistance and gluconeogenesis. New evidence demonstrates that the elevation may induce lipogenesis, purine biosynthesis and gluconeogenesis through activation of transcription factors of ChREBP and NRF2. In cancer cells, NADH/NAD^+ elevation under the Warburg effect is primarily derived from decreased NADH consumption in the mitochondrial respiration. Alternatively, NRF2 overactivation from gene mutation represents another mechanism of NADH/NAD^+ elevation from NADH production in the cancer cells. The elevation is required for quick proliferation of cancer cells through induction of biosynthesis of the essential molecules. It appears that the causes of reductive stress are different between obesity and cancer, while its impact in anabolism is similar in the two conditions.

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Reductive stress is opposite to oxidative stress. Reductive stress has been investigated in cancer cells in the context of tumor metabolism, especially in the study of mechanism and impact of Warburg effect. Oxidative stress usually comes from over production of reactive oxygen species (ROS) from activation of the mitochondrial respiration[1]. However, reductive stress is often a consequence of NADH accumulation derived from inhibition of HAND consumption by mitochondrial respiration leading to the elevated NADH/NAD^+ ratio[2,3]. The tumor-associated reductive stress drives anabolism required for cell proliferation. Emerging evidence suggests that reductive stress is also a metabolic feature in obesity underlying the metabolic disorders[4-6]. However, significance of the reductive stress remains to be established in obesity.

Reductive stress is considered a metabolic feature of cancer cells associated with "Warburg effect", in which a higher NADH/NAD^+ ratio (a high level of NADH) is established from constraining of NADH consumption in the mitochondrial respiration[2,3]. The biological significance of reductive stress is to promote biosynthesis of lipids, nucleotides and amino acids in support of cell proliferation in tumors[2,3]. Although the reductive stress is found in obesity[4-6], the biological significance remains largely unknown[7]. Early studies suggest that obesity is associated with oxidative stress across various tissues/organs, contributing to metabolic disorders. In the adipose tissue, the oxidative stress exacerbates inflammation and insulin resistance[8]. In the liver, the oxidative stress involves in the development of non-alcoholic fatty liver disease and insulin resistance[9]. In addition to the oxidative stress, reductive stress is another metabolic feature of obesity as suggested by NADH elevation in the liver of obese mice and human[4-6]. However, the molecular mechanism by which NADH contributes to the metabolic disorder is unknown. Two exciting studies published in 2024 has shed light on the mechanism in the study of phenotypes of liver-specific gene knockout mice. In the first study, NADH elevation is found to promote hepatic de novo lipogenesis through a transcriptional pathway by activation of ChREBP (carbohydrate response element binding protein)[10]. In the second study, NADH elevation is found to enhance hepatic glucose production through a transcriptional mechanism by activation of NRF2 (nuclear respiratory factor 2)[11]. Those studies suggest that signaling activities of NADH involve in activation of transcription factors. The finding may apply to tumor cells for understanding of the mechanism underlying the proliferation-associated anabolism. The studies provide clues to the molecular mechanism for reductive stress in the obesity-associated metabolic disorders, such as hyperlipidemia and hyperglycemia.

NADH, synthesized abundantly through multiple catabolic pathways within cells, plays a crucial role in energy production by mitochondria. NADH is elevated in several physiological and pathological conditions (Fig. 1). NADH is found as a driving force in anabolism for cell growth (such as tumor and stem cells) as well as repair[2,3]. NADH is produced primarily from glycolysis, the tricarboxylic acid (TCA) cycle and β-oxidation of long-chain fatty acids. Glucose is metabolized into pyruvate in glycolysis with NADH production, and pyruvate is then converted into acetyl-CoA in mitochondria. NADH and $FADH_2$ are efficiently produced during acetyl-CoA breakdown in the TCA cycle. These molecules (NADH/$FADH_2$) are utilized by the respiratory chain (ETC) for ATP synthesis through oxidative phosphorylation in mitochondria[12-14,15]. NADH/$FADH_2$ are consumed largely by the mitochondrial respiration, in which they donate electrons for energy release in support of the mitochondrial potential. Interestingly, NADH is essential for de novo lipogenesis in the cytosol, where it acts as a reducing agent to convert carbon dioxide (CO_2) to a carboxyl group, ultimately facilitating the synthesis of saturated fatty acids. Elevation of NADH/NAD ratio in cells, observed under conditions, such as the "Warburg effect" in cancer cells[16], leads to biosynthesis of materials (such as nucleotides, amino acids, and lipids) for proliferation and survival of cancer cells. Overall, NADH serves as a critical substrate in the catabolic processes, supporting energy (ATP) production, and also in the anabolic processes, facilitating cell growth and repair through biosynthesis of relevant molecules. NADH regulates glucose metabolism including glycolysis, gluconeogenesis, and TCA cycle[17,18].

In addition to NADH, other metabolites such as NADPH and glutathione (GSH), involve in the maintenance of oxidative/reductive balance within cells. NADPH, generated primarily through the pentose phosphate pathway, acts as a key reducing equivalent in anabolic reactions, including fatty acid synthesis and the detoxification of reactive oxygen species (ROS). It also provides the reducing power by regeneration of reduced GSH from its oxidized form (GSSG), which is catalyzed by glutathione reductase. GSH serves as a major antioxidant that neutralizes ROS in redox homeostasis. Specific proteins, such as glutathione peroxidase and peroxiredoxins, utilize GSH and NADPH to detoxify peroxides in protection of cells from oxidative damage. These molecules, along with NADH, constitute the reductive force in the control of oxidative stress[1].

NADH plays a significant role in nucleic acid synthesis, particularly under conditions of elevated NADH/NAD^+ ratio to provide the reducing power required for nucleotide synthesis[19]. This process is essential for biosynthesis of the backbone of nucleic acid molecules. Furthermore, NADH activates key enzymes involved in DNA and RNA production, including DNA polymerases and DNA ligases. These enzymes are integral to various stages of nucleic acid synthesis, such as DNA replication and repair, which are crucial for maintaining genome stability and ensuring the accurate transmission of genetic information during cell division in the physiological conditions. The NADH-mediated nucleic acid biosynthesis may have significant implications in pathological conditions. For example, in the cancer cells, elevated NADH levels can support rapid cell proliferation by facilitating increased nucleic acid synthesis.

The quick progress in the study of reductive stress is dependent on development of new transgenic systems. The NADH/NAD^+ ratio may be induced in cells by expression of a pyridine nucleotide transhydrogenase from *Escherichia coli* (soluble transhydrogenase, EcSTH), which catalyze hydride transfer from NADPH to NAD^+ to generate NADH, which is coupled with an increase in NADH/NAD^+ ratio and a reduction in the NADPH/$NADP^+$ ratio. Expression of EcSTH in mitochondria leads to robust elevation of NADH/NAD^+ ratio in mitochondria of mammalian cells[20]. The system has led to the finding that reductive stress promotes nucleic acid biosynthesis. Using the EcSTH system, Yang et al.[19] found that NADH/NAD^+ ratio elevation was found to promote nucleic acid (purine) biosynthesis to provoke a massive energy consumption resulting in cell death. Blocking the purine biosynthesis prevented NADH accumulation-associated cell death[19].

Moreover, the NADH/NAD^+ ratio may be reduced by expression of LbNOX gene, which encodes an enzyme catalyzing conversion of NADH into NAD^+ through production of water[21]. Given that the mitochondrial respiration is a major metabolic pathway in consumption of NADH in most cell types, inhibition of the mitochondrial activity (such as under hypoxia) is associated with NADH elevation and thus reductive stress[22]. The

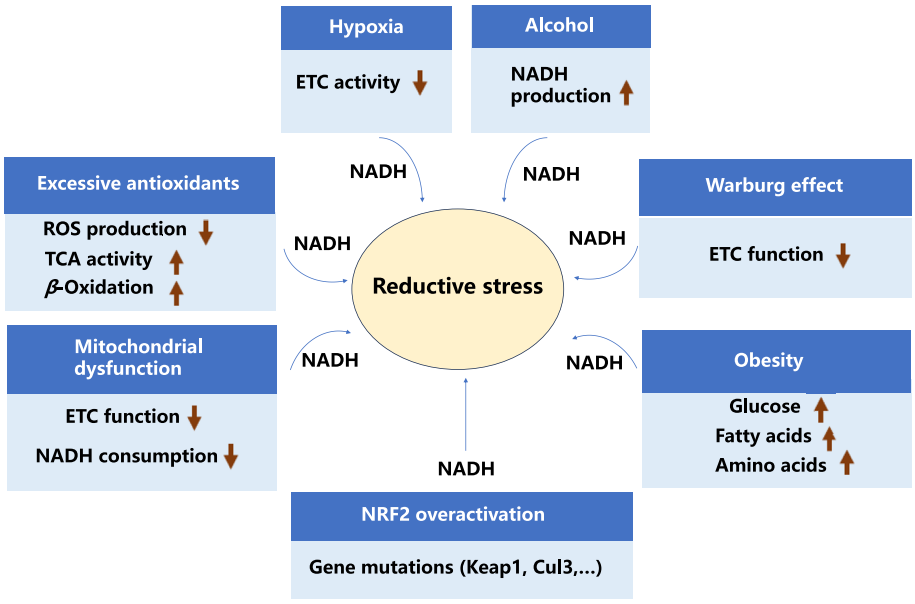

Figure 1 Physiological and pathophysiological conditions that increase NADH accumulation in cells for reductive stress. Restriction of mitochondrial respiration by hypoxia and mitochondrial dysfunction leads to NADH elevation due to reduced NADH consumption by the electron transfer chain (ETC). In contrast, an increase in NADH production under conditions of alcohol intake, obesity (substrate overloading), NRF2 activation, induction of TCA and β-oxidation together with low ROS production leads to NADH elevation. In cancer cells, restriction of the respiratory chain activity in mitochondria leads to NADH accumulation from less NADH consumption, and NRF2 super-activation from gene mutations (Keap1 or Cul3) leads to NADH over production.

mitochondrial function is restrained in cancer cells contributing to the maintenance of reductive stress environment for cell proliferation[2]. The reductive stress drives biosynthesis of materials in cancer cells for metabolic reprogram during cell proliferation[23]. However, in non-proliferating cells (such as normal differentiated cells), the reductive stress from mitochondrial inhibition leads to cell death through cell toxicity. The toxicity may be reduced by expression of LbNOX in the cytosol or mitochondria[21].

In obesity, the reductive stress may contribute to lipid disorders through an activity in the liver. NADH rising for elevated NADH/NAD^+ ratio has been reported in the liver[4-6], which is likely a result of mitochondrial overloading from energy surplus. The NADH oversupply triggers reductive stress in hepatocytes, which in turn may promote lipid biosynthesis for the obesity-associated hepatic steatosis. The molecular mechanism of NADH activity involves in activation of gene transcription. One mechanism is NADH inhibition of the transcription co-repressor SIRT1 (sirtuin-1), which is a NAD-dependent deacetylase. The mechanism has been extended by recent identification of relationship of NADH and ChREBP[10], a transcription factor. In the study, NADH is found to be activated ChREBP in hepatocytes, converting excess carbohydrates into fat through transcriptional activation of genes responsible for *de novo* lipogenesis in the liver. The conclusion is supported by observations under four conditions[10]: LbNOX expression to lower NADH/NAD^+ ratio, EcSTH expression to raise NADH/NAD^+ ratio, ethanol gavage to increase NADH/NAD^+, and manipulation of extracellular lactate/pyruvate ratio to equilibrate with cytosolic NADH/NAD^+[10]. Consequently, the liver increased fat export in the form of very low-density lipoprotein leading to hyperlipidemia, a condition characterized by high levels of fats and cholesterol in the bloodstream. The study demonstrates that NADH is able to promote lipogenesis through activation of ChREBP in the cell adaptation to reductive stress.

Reductive stress has a significant activity in the regulation of gluconeogenesis, which is essential for maintenance of blood glucose homeostasis. Gluconeogenesis occurs primarily in the liver and kidneys by consumption of non-carbohydrate substrates, such as amino acids, glycerol and lactate. This pathway is typically activated during fasting or prolonged food deprivation to supply glucose to the vital organs, such as the brain and red blood cells. The gluconeogenesis is coupled with inhibition of fatty acid synthesis (lipogenesis) in the liver as a consequence of decreased insulin level. After meals, gluconeogenesis is inhibited by insulin along induction of lipogenesis in the liver. In obesity, the surplus NADH is associated with enhanced gluconeogenesis in liver in the presence of insulin resistance[4-6]. The NADH activity receives new support in a recent study, in which NADH elevation was observed in the liver-specific double ACC1/2 knockout mice, and the alteration was found to induce hepatic insulin resistance[11]. In the study, NADH was found to promote gluconeogenesis and lipogenesis together[11], which are often observed in obesity for the high risk of type 2 diabetes.

Recent studies have enforced the role of NRF2 in regulating the reductive stress. Activation of NRF2 leads to the upregulation of aldehyde dehydrogenase 3 family member A1, resulting in NADH-mediated reductive stress, which was observed in cancer cells[24]. Research conducted by Andrew G. Manford and colleagues reveals a cellular mechanism aimed at detecting and alleviating reductive stress. They elucidate how reductive stress targets specific cysteine residues in folliculin interacting protein 1 (FNIP1), thereby affecting mitochondrial function and redox balance. Interactions among proteins such as E3 ligase $CUL2^{FEM1B}$ and FNIP1, with zinc playing a pivotal role in the response to reductive stress[25]. A recent study by Gu et al.[26] highlights the complex role of NRF2 and CRL3 in hepatic metabolism under obese conditions. The study shows that

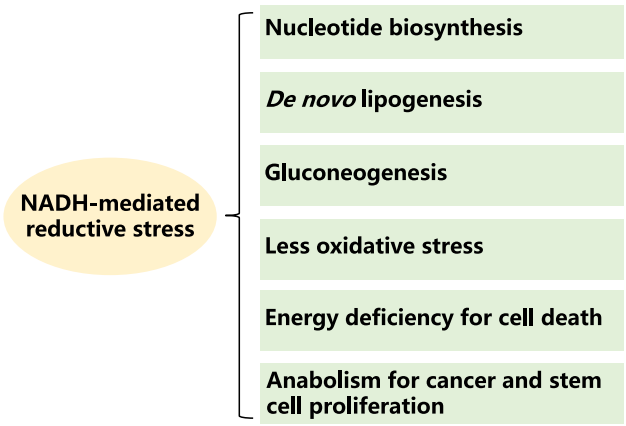

Figure 2 NADH mediates metabolic reprogramming, cell death and tumor growth under reductive stress in cells through impact in several anabolism pathways. NADH has been reported to promote biosynthesis of nucleotide, fatty acids and glucose, which are required for cell proliferation in cancer cells and stem cells. In non-dividing cells, the biosynthesis may cause energy deficiency for cell death.

knockout of hepatocyte Cul3 in obese mice reduces hepatic steatosis, but triggers systemic metabolic disturbances due to NRF2 overactivation. The overactivation leads to NADH-mediated reductive stress, disrupting lipid metabolism and exacerbating insulin resistance. These findings bring new insight into mechanisms of the reductive stress in obesity.

Cells employ various mechanisms to alleviate NADH-associated reductive stress, such as lactate synthesis and ETC consumption of NADH (Fig. 2). Through lactate dehydrogenase, cells convert pyruvate to lactate, effectively decreasing NADH by regenerating NAD^+ and thus mitigating reductive stress. Similarly, the mitochondrial ETC consumes NADH to generate NAD^+ during oxidative phosphorylation, which is crucial in prevention of NADH surplus. In cancer cells, however, reductive stress from NRF2 over-activation is often exacerbated by gene mutations, such as Keap1 and Cul3 mutations. The mutations lead to NRF2 overactivation independent of the Warburg effect for excessive NADH accumulation in the metabolic reprogramming in favor of tumor growth[27,28]. The studies suggest NADH as a potential molecular target in cancer therapy. Selective modulation of NADH levels in cells could help to correct metabolic imbalances in obesity and diabetes, or restraining the proliferative capacity of cancer cells by disrupting their redox homeostasis.

In summary, reductive stress, driven by the elevated NADH/NAD^+ ratio, promotes the biosynthesis of nucleotides, glucose, and fatty acids (Fig. 2). This process is essential for the proliferation of cancer cells and stem cells by synthesis of building blocks in cell proliferation. Emerging evidence suggests that reductive stress may be a key mechanism underlying metabolic disorders in obesity and type 2 diabetes. Recent studies have illuminated NADH activity in the liver of obese models, and NADH may regulate gene transcription by activation of transcription factors, such as ChREBP and NRF2. Emerging evidence suggests that reductive stress may be a metabolic feature shared by both obesity and cancer. However, the mechanisms are likely different in the formation of reductive stress in two conditions. In obesity, the stress may be a result of mitochondrial overloading with substrates (such as glucose, fatty acids and amino acids). In cancer, the reductive stress is derived from restraining of mitochondrial respiration or NRF2 overactivation from gene mutation. Current evidence provides a proof of concept of reductive stress in cancer and obesity. Moving forward, research should focus on testing the possibilities further. Additionally, translation of the findings into clinical practice is crucial, particularly in developing safe and effective strategies to modulate NADH/NAD^+ ratios without adverse effects. Bridging the gap between laboratory research and clinical practice will be essential in managing the reductive stress in disease treatment.

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Author contributions

Man Luo: Writing − original draft. Xiwen Ma and Jianping Ye: Writing − review & editing, Conceptualization.

Conflicts of interest

The authors declare no conflict of interest.

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