Sources and implications of NADH/NAD⁺ redox imbalance in diabetes and its complications

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Abstract: NAD⁺ is a fundamental molecule in metabolism and redox signaling. In diabetes and its complications, the balance between NADH and NAD⁺ can be severely perturbed. On one hand, NADH is overproduced due to influx of hyperglycemia to the glycolytic and Krebs cycle pathways and activation of the polyol pathway. On the other hand, NAD⁺ can be diminished or depleted by overactivation of poly ADP ribose polymerase that uses NAD⁺ as its substrate. Moreover, sirtuins, another class of enzymes that also use NAD⁺ as their substrate for catalyzing protein deacetylation reactions, can also affect cellular content of NAD⁺. Impairment of NAD⁺ regeneration enzymes such as lactate dehydrogenase in erythrocytes and complex I in mitochondria can also contribute to NADH accumulation and NAD⁺ deficiency. The consequence of NADH/NAD⁺ redox imbalance is initially reductive stress that eventually leads to oxidative stress and oxidative damage to macromolecules, including DNA, lipids, and proteins. Accordingly, redox imbalance-triggered oxidative damage has been thought to be a major factor contributing to the development of diabetes and its complications. Future studies on restoring NADH/NAD⁺ redox balance could provide further insights into design of novel antidiabetic strategies.

Keywords: mitochondria, complex I, reactive oxygen species, polyol pathway, poly ADP ribosylation, sirtuins, oxidative stress, oxidative damage

Introduction

Chronic elevation of blood glucose, known as diabetic hyperglycemia, is a hallmark of diabetes mellitus.¹⁻⁴ This persistent hyperglycemia can lead to long term damage to tissues such as the kidney, eyes, nerves, blood vessels, and heart. ³⁻⁶ For non-insulin-dependent tissues, a high level of blood glucose would mean a high level of glucose metabolism as glucose entry into the cells is not limited by insulin deficiency.⁷⁻⁸ Since one of the major purposes of glucose metabolism is to provide electrons that are stored mainly in NADH and FADH₂ for ATP production via the processes of glycolysis and mitochondrial metabolic pathways, NADH would be in an oversupply state when glucose overload occurs. This excess NADH can break the redox balance between NADH and NAD⁺, and eventually can lead to oxidative stress and a variety of metabolic syndromes.⁹⁻¹³ Hence, it suffices to say that diabetes is a redox imbalance disease.¹⁴,¹⁵

In this review, we delineate the sources and the pathways that contribute to NADH/NAD⁺ redox imbalance, and the potential consequences of this redox imbalance in diabetes. Regarding pathways that contribute to NADH/NAD⁺ redox imbalance, we focus on both the conventional glucose metabolic pathways and polyol pathway that get
activated by high level of blood glucose.\textsuperscript{16–18} We also discuss
the pathways that utilize NAD\textsuperscript{+} as substrates such as sirtuins
deacetylation pathways\textsuperscript{19,20} and poly ADP ribosylation path-
way.\textsuperscript{21,22} Additionally, NADH/NAD\textsuperscript{+}-recycling enzymes such
as lactate dehydrogenase (LDH) and mitochondrial complex I (NADH-ubiquinone oxidoreductase\textsuperscript{23,24}) are also discussed.
We believe that the consequences triggered by NADH/NAD\textsuperscript{+}
redox imbalance are eventually reflected by oxidative stress
and cell death that are known to be involved in the patho-
genesis of diabetes and its complications.

**NADH production by the conventional glucose metabolic pathways**
The pair of NADH and NAD\textsuperscript{+} plays a crucial role in metabo-
lism and redox signaling.\textsuperscript{25–30} The central pathways involved
in complete glucose breakdown and electron storage in
NADH are the glycolytic pathway and the Krebs cycle. As
shown in Figure 1, glyceraldehyde 3-phosphate dehydroge-
nase in the glycolytic pathway makes NADH from NAD\textsuperscript{+}.
This is followed by pyruvate dehydrogenase complex that
also makes NADH from NAD\textsuperscript{+}, whereby the actual enzyme
catalyzing NADH formation is dihydrolipoamide dehydro-
ge Germans.\textsuperscript{31,32} After acetyl-CoA enters into the Krebs cycle, more
molecules of NADH are produced, which can be ascribed
to the action of isocitrate dehydrogenase, \(\alpha\)-ketoglutarate
dehydrogenase, and malate dehydrogenase, respectively.
Fatty acid \(\beta\)-oxidation fueling the production of acetyl-CoA
can also be a significant source of NADH.\textsuperscript{33} Additionally,
glutamate dehydrogenase, a central enzyme involved in
\(\alpha\)-ketoglutarate formation from glutamate,\textsuperscript{34} can also make
NADH from NAD\textsuperscript{+}.\textsuperscript{35,36} Under hyperglycemic conditions,
both the glycolytic pathway and the Krebs cycle can be
intensively fluxed by glucose.\textsuperscript{37} Therefore, NADH can be
overproduced in diabetes via these pathways,\textsuperscript{38} and excess
NADH is known to cause reductive stress.\textsuperscript{13,39–43}

**NADH production by polyol pathway**
The polyol pathway, as shown in Figure 2, involves two con-
secutive reactions that are catalyzed by aldose reductase and
sorbitol dehydrogenase, respectively. This pathway is usually
rather inactive under euglycemic condition\textsuperscript{16} but can become
a highly active glucose disposal pathway under diabetic
hyperglycemic condition.\textsuperscript{44,45} The major feature of this path-
way is the production of NADH, sorbitol, and fructose.\textsuperscript{16,46–48}
Each of these intermediates or products plays a role in the
pathogenesis of diabetes and its complications.\textsuperscript{16,46–49}
For example, sorbitol can accumulate in retinal and renal tissues
and causes osmotic stress and cell death,\textsuperscript{50,51} and fructose
can cause nonenzymatic protein glycation or nitration\textsuperscript{52,53}
and contributes to pathogenesis of nonalcoholic fatty liver
disease.\textsuperscript{54} More importantly, a massive NADH production by
this pathway is known to perturb redox imbalance between
NADH and NAD\textsuperscript{+}, and consumption of NADPH can impair
the function of glutathione reductase, leading to accumulation
of oxidized form of glutathione and further accentuation of
redox imbalance.\textsuperscript{13,55} As such, inhibition or deletion of aldose
reductase, a rate-limiting enzyme in the polyol pathway, has
been demonstrated to be antidiabetic.\textsuperscript{56–60}

**NAD\textsuperscript{+}-degradation pathways**
NAD\textsuperscript{+} is not only an electron acceptor but can also serve
as a substrate and be degraded during enzyme-catalyzed
reactions. Two major enzymatic pathways that use NAD\textsuperscript{+} as
their substrate are sirtuins and poly ADP ribose polymerases
(PARP).\textsuperscript{27,61} As shown in Figure 3A, sirtuins use NAD\textsuperscript{+}
for their deacetylation reactions, whereby NAD\textsuperscript{+} is degraded
and nicotinamide and 2’-O-acetyl-ADP ribose are formed.
Sirtuins are inducible enzymes.\textsuperscript{62,63} Therefore, if NAD\textsuperscript{+} level

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**Figure 1 Metabolic pathways and enzymes involved in NADH production using NAD\textsuperscript{+} as their cofactor.**

**Notes:** The enzymes are glyceraldehyde 3-phosphate dehydrogenase in the glycolytic pathway, pyruvate dehydrogenase complex catalyzing the formation of acetyl-CoA from pyruvate, and the isocitrate dehydrogenase, \(\alpha\)-ketoglutarate
dehydrogenase, and malate dehydrogenase in the Krebs cycle. Additionally, fatty acid oxidation that yields one molecule of NADH per one molecule of acetyl-CoA produced is also shown.
Redox imbalance in diabetes

is low, sirtuin protein content would be low. As acetylated proteins usually exhibit impaired functions, deacetylation by sirtuins usually improve the function of the target proteins. Therefore, sirtuins can be activated by starvation or caloric restriction to safeguard cell survival. On the other hand, overnutrition such as in diabetes that usually produces excess NADH with diminished NAD⁺ content can often lead to attenuation of sirtuin protein content. Therefore, enhancing sirtuin expression in diabetic tissues has been suggested as a therapeutic approach for treating diabetes and its complications. It should be noted that among the seven members of the sirtuin family, sirtuin 4 does not possess deacetylation activity but rather exhibits mono- or poly ADP ribosyltransferase activity.

**Figure 2** Polyol pathway.
**Notes:** Shown are the two reactions catalyzed, respectively, by aldose reductase and sorbitol dehydrogenase. The pathway makes sorbitol from glucose, fructose from sorbitol, and NADH from NADPH via NAD⁺. Sorbitol can trigger osmotic stress and cell death; fructose can induce nonenzymatic glycation or contributes to nonalcoholic fatty liver disease. NADH can cause reductive stress that eventually leads to oxidative stress. Additionally, NADPH depletion can also impair glutathione reductase resulting in accumulation of oxidized glutathione that can further impair cellular redox balance.

**Abbreviations:** GSSG, oxidized glutathione; GSH, reduced glutathione.

**Figure 3** Two enzyme systems that are involved in NAD⁺ degradation.
**Notes:** (A) Sirtuins that catalyze protein deacetylation using NAD⁺ as a substrate. (B) Poly ADP ribose polymerase that catalyzes protein poly ADP ribosylation at the cost of NAD⁺. In both reactions, nicotinamide is formed.

**Abbreviation:** 2'-O-acetyl-ADPR, 2'-O-acetyl-ADP ribose.
While numerous studies demonstrate that elevating sirtuin protein content, such as that of sirtuin 3, ameliorates diabetes and its complications,67,75–77 a question arising is that whether it is possible that elevated levels of sirtuins consume more NAD+ and make the redox imbalance situation worse. This seemingly is not the case. It is probable that elevated levels of sirtuins alter the profiles of a given acetylated/deacetylated proteome, rendering metabolic pathways more efficient, which leads to more NADH utilization and thus more NAD+ regeneration.74 It has been reported that deacetylation by sirtuin protein can enhance NADPH production, which may be involved in restoring cellular redox balance.78 Nonetheless, whether elevation of sirtuin levels in diabetes could restore or improve NADH/NAD+ redox balance needs to be further thoroughly investigated.

Another enzyme system that consumes and degrades NAD+ is PARPs, especially PARP-179 that can be activated by DNA damage.22,80 As shown in Figure 3B, the products of PARP-catalyzed reaction are poly ADP ribosylated proteins and nicotinamide derived from NAD+. The problem caused by activation of PARP in diabetes is that the enzyme is often overactivated,81–83 resulting in potential depletion of NAD+, which would further perturb NADH/NAD+ redox balance, leading to cell death.21,79,84–85 PARP has been touted as a promising target for antidiabetic therapy. Indeed, knocking out or knocking down PARP expression can prevent animals from developing diabetes.86–88 Drugs that inhibit PARP activity have also been developed and tested for antidiabetic therapy.89–93 For example, 1,5-isoquinolinediol as a PARP inhibitor has been shown to improve corneal epithelial innervation in diabetic rats,94 and PARP inhibition could improve erectile function in diabetic rodents.95

Regeneration of NAD+ from NADH

For metabolism to continue, NAD+ has to be regenerated from NADH. There are two major pathways that can achieve this task, namely, LDH and mitochondrial complex I that is the first electron entry point in the electron transport chain.96–98 In anaerobic metabolism such as in erythrocytes where no mitochondria exist, LDH is responsible for NAD+ regeneration (Figure 4A). Under aerobic condition, however, mitochondrial complex I is responsible for NAD+ regeneration (Figure 4B). Hence, it is imaginable that NADH oversupply could overwhelm LDH or complex I.101 Indeed, it has been shown that diabetic hyperglycemia increases the enzyme activity of LDH in red blood cells and in small platelets to handle NADH over-influx.102,103 On the other hand, changes in complex I function in diabetes and its complications remain very sketchy. Nonetheless, it has been reported that complex I activity is decreased in diabetic skeletal muscles104 but increased in diabetic kidneys.105 Therefore, it seems that changes in complex I activity are tissue dependent in diabetic subjects. It would be interesting to survey complex I activity from tissue to tissue in diabetic rodents or possibly humans.

Detrimental effects of redox imbalance in diabetes

When excess NADH accumulates, the enzymes that produce NADH from NAD+ will be inhibited. For example, both glyceraldehyde 3-phosphate dehydrogenase and dihydrolipoamide dehydrogenase in the pyruvate dehydrogenase complex can be inhibited by NADH,106,107 leading to potential reactive oxygen species (ROS) production.82,108,109 Moreover, mitochondrial electron transport chain can be overloaded by this electron donor.110 The direct pressure of this NADH overload would be on complex I, which is a major site for generation of ROS.111–116 The feature of this NADH overloading in complex I is that the major site for aerobic NAD+ regeneration.

Figure 4 Major cellular enzymes involved in NAD+ regeneration.

Notes: Shown are (A) lactate dehydrogenase in red blood cells or under hypoxic conditions and (B) mitochondrial complex I that is the major site for aerobic NAD+ regeneration.

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The observation that complex I ROS production plays a role in improving redox balance.158–163 High levels of NADH can cause oxidative stress by promoting the generation of ROS.164 While drugs inhibiting aldose reductase in the polyol pathway164,165 or PARP166,167 will continue to remain as active areas of investigation in the future, NAD+ regeneration enzymes such as complex I should also be studied168 to provide insights into how excess NADH can be oxidized under glucose overload conditions. Additionally, administration of NAD+ precursors or analogs166,167 can also serve as an approach to treating diabetes and its complications. The ultimate goals of all these prospective studies are to restore NADH/NAD+ redox balance in diabetes and its complications for therapeutic purposes.

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**Disclosure**

The authors report no conflicts of interest in this work.

**References**


