

Oxidative stress and diabetes: antioxidative strategies

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Abstract Diabetes mellitus is one of the major public health problems worldwide. Considerable recent evidence suggests that the cellular reduction–oxidation (redox) imbalance leads to oxidative stress and subsequent occurrence and development of diabetes and related complications by regulating certain signaling pathways involved in β -cell dysfunction and insulin resistance. Reactive oxide species (ROS) can also directly oxidize certain proteins (defined as redox modification) involved in the diabetes process. There are a number of potential problems in the clinical application of antioxidant therapies including poor solubility, storage instability and non-selectivity of antioxidants. Novel antioxidant delivery systems may overcome pharmacokinetic and stability problem and improve the selectivity of scavenging ROS. We have therefore focused on the role of oxidative stress and antioxidative therapies in the pathogenesis of diabetes mellitus. Precise therapeutic interventions against ROS and downstream targets are now possible and provide important new insights into the treatment of diabetes.

Keywords diabetes; oxidative stress; redox modification; antioxidative therapy; novel antioxidant delivery

Introduction

Diabetes mellitus is a chronic disease characterized by hyperglycemia resulting from decreased insulin secretion or insulin resistance, which leaves the body incapable of responding fully to insulin. The worldwide diabetes mellitus epidemic affected 425 million people in 2017, and the number of people with diabetes is expected to increase to 629 million by 2045 (International Diabetes Federation, 2017). There are three widely accepted major forms of diabetes including type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) and gestational diabetes mellitus (GDM), among which T2DM accounts for approximately 90% of all cases of diabetes [1–3]. In addition, there are other less common types including monogenic diabetes, an inherited form of diabetes [4], and cystic fibrosis-related diabetes (CFRD). CFRD is a unique type of diabetes that is common in patients with cystic fibrosis (CF). If not well managed, all cases of diabetes may develop diabetic complications which are the major causes of high mortality and disability.

Increasing studies suggest that oxidative stress plays a

pivotal role in the pathogenesis and progression of diabetes. Oxidative stress was observed in experimental diabetes as early as 1982 [5], and has been found to play an important role in all cases of diabetes mellitus (particularly T2DM) and the pathogenesis of diabetic complications. Nevertheless, the precise underlying mechanisms are not yet fully understood. T2DM is associated with increased oxidative stress resulting from several abnormalities, including hyperglycemia, inflammation and dyslipidemia [6,7]. In turn, elevated reactive oxide species (ROS) can act as a second messenger and regulate the biological function of various proteins including I κ B kinase β (IKK β), protein kinase C (PKC) and Kelch-like ECH-associated protein 1 (Keap1) through interaction with cysteine residues (termed “redox sensors”) of these proteins [8,9]. This dynamic modification of intracellular redox sensors by ROS is defined as redox modification, similar to other posttranslational modifications such as protein phosphorylation, acetylation, or ubiquitination, which plays an important role in the development of diabetes [10]. Redox modification of these proteins can activate alternative downstream signaling pathways which play critical roles in impaired insulin secretion and insulin resistance, facilitating the development of diabetes and diabetic complications.

Because of the intimate and complicated association between oxidative stress and diabetes, multiple attempts have been made to improve the health of patients with diabetes by dietary antioxidant supplements, such as

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enzymatic antioxidants-like mimics, vitamin C, and vitamin E. However, the clinical failure of antioxidant therapies to date is controversial, and, even worse, some studies show that antioxidants may be harmful and even increase mortality due to non-selective scavenging of ROS. Excessive clearance of ROS may impair some essential intracellular signaling and metabolic functions associated with ROS. On the other hand, increasing studies are suggesting that antioxidant inefficiency may also be mainly due to its poor solubility, permeability, storage instability, first-pass metabolism or gastrointestinal degradation [11]. Thus it is necessary to develop novel delivery approaches for antioxidants and specific drugs targeting oxidative stress and redox modification. In addition, physiologic approaches (such as healthy diet, physical activity) have been a prerequisite of managing and preventing diabetes and end-organ damage.

Overview of diabetes mellitus

Diabetes mellitus is characterized by the elevation of glucose in blood (hyperglycemia) due to insufficiency in production and/or action of endogenous insulin [12]. Insulin is an essential hormone secreted by β -cell of pancreas, prompting cells to absorb glucose from the bloodstream. A relative or absolute deficiency of insulin resulting from β -cell dysfunction and insulin resistance (IR) causes hyperglycemia. Without the belated management, abnormal glycemic regulation may augment the risk of microvascular complications (neuropathy, retinopathy, and nephropathy), macrovascular complications (angina, stroke, coronary artery diseases (CADs), myocardial infarction, congestive heart failure and peripheral artery disease (PAD)) and both micro- and macrovascular complication (diabetic foot) [13–15]. In addition, some evidence suggests subjects with diabetes mellitus have increased risk of physical and cognitive disability, cancer, tuberculosis and depression [16–21].

Type 1 diabetes mellitus

T1DM or IDDM (insulin-dependent diabetes mellitus) is considered to be an autoimmune disease in which β -cells are destroyed. This most commonly happened in children and adolescents. The precise mechanisms of T cell-mediated β -cells destruction remain largely unknown, but genetic susceptibility (class II HLA alleles) and environmental triggers (some dietary factors, viral infection and toxins) have been implicated [22–25]. When the symptomatic onset of T1DM is perceived, more than 85% of β -cell mass has been destructed, leading to deficiency of insulin and subsequent elevation of blood glucose. Importantly, hyperglycemia enhances the production of ROS which in turn lowers insulin secretion and action [26].

Type 2 diabetes mellitus

T2DM, also known as insulin-independent diabetes mellitus (NIDDM), is characterized by hyperglycemia caused by several pathological processes such as decreased secretion of insulin, abnormal incretin effect and excessive cell glucagon production and insulin resistance (IR). The prominent defect of T2DM is insulin resistance that impairs the action of insulin. In the early stages of T2DM, increasing production of insulin is indispensable to maintain the blood glucose level to overcome IR. Nevertheless, with the progression of T2DM, enhanced insulin secretion fails to compensate for insulin resistance [27]. It has been reported that obesity, overweight, genetic component (myosin heavy chain genes), sedentary life style and old age are possible relevant factors for type 2 diabetes [28–30]. Furthermore, compelling evidence has shown that oxidative stress results in more severe T2DM by decreasing both insulin secretion and function and causes many microvascular and macrovascular complications due to the damage of DNA, proteins and or lipids [31].

Gestational diabetes mellitus

GDM refers to the slight hyperglycemia which is first detected during the second and third trimesters of pregnancy (International Diabetes Federation, 2017). Pregnant women with GDM and their offspring both have a higher risk of developing type 2 diabetes [32–35]. GDM is a metabolic complication of pregnancy and is characterized by glucose intolerance and insulin resistance associated with hormone secretion from the placenta, obesity, family history, increased inflammation, and oxidative stress in the placenta and fetus [36–38]. Recent studies have shown that the decompensation for oxidative stress may play a critical role in GDM by decreasing insulin sensitivity index [39,40].

Free radicals and antioxidants

Free radicals are metabolic byproducts and ephemeral reactive chemical entities containing one or more unpaired electrons. There are various forms of free radicals such as ROS including hydroxyl ($\cdot\text{OH}$), superoxide ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydrochlorous acid (HOCl) as well as RNS (reactive nitrogen radicals) including nitric oxide (NO), nitrogen dioxide (NO_2), and the non-radical peroxynitrite (ONOO^-) [41], all of which are implicated in diabetes and diabetic complications (Fig. 1). Low to moderate levels of free radicals are necessary to regulate multiple cellular physiologic activities (such as responses to anoxia, anti-infection, and mitosis) [42], while excessive levels of ROS and RNS lead to oxidative stress and

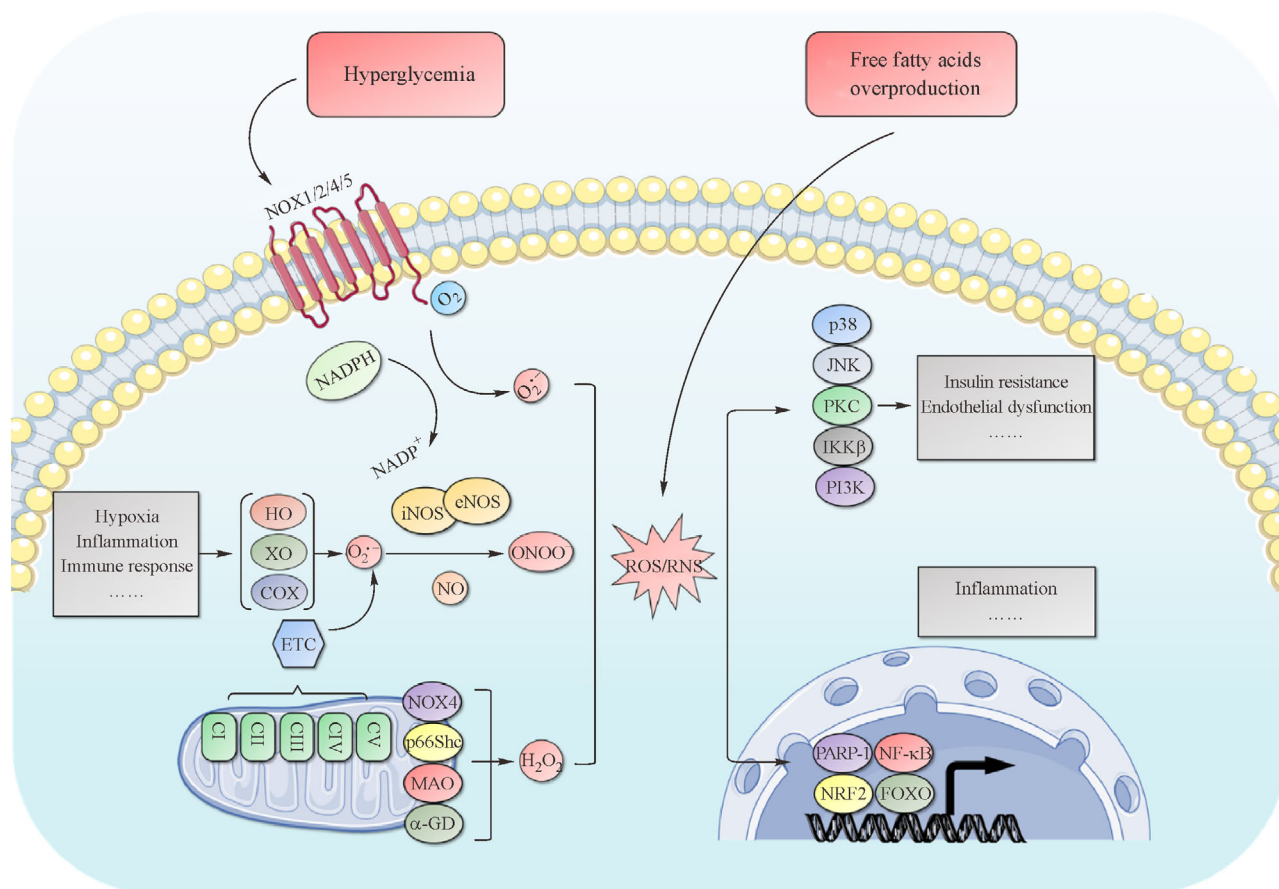


Fig. 1 The sources of ROS/RNS and their harmful effects. ROS/RNS arise from mitochondrial electron transport chain or/and non-mitochondrial pathways. When cells and tissues are exposed to hypoxia, inflammation and immune response, particularly hyperglycemia, and high free fatty acids, the generation of ROS/RNS will be elevated. The overproduction of ROS/RNS leads to oxidative stress that regulates important cell signaling pathways which govern cell proliferation, inflammation, and cell survival. Abbreviations: NOX, nicotinamide adenine nucleotide phosphate oxidase; NADPH, nicotinamide adenine nucleotide phosphate; $O_2^{\bullet-}$, superoxide; HO, hemeoxygenase; XO, xanthine oxidase; COX, cyclooxygenases; iNOS, inducible NOS; eNOS, endothelial NOS; NOS, nitric oxide synthase; ONOO⁻, peroxynitrite; NO, nitric oxide; ETC, electron transport chain; CI, complex I; MAO, monoamine oxidase; α -GD, α -glycerophosphate dehydrogenase; H_2O_2 , hydrogen peroxide; ROS, reactive oxygen species; RNS, reactive nitrogen species; JNK, c-jun N-terminal kinase; PKC, protein kinase C; IKK β , I κ B kinase β ; PI3K, phosphatidylinositolide 3-kinase; PARP-1, poly (ADP-ribose) polymerases; NF- κ B, nuclear transcription factor κ B; Nrf2, nuclear factor E2-related factor 2; FOXO, forkhead box protein O.

subsequent damage of biomacromolecules. For example, inhibiting phosphorylation sites of endothelial nitric oxide synthase (eNOS), a major nitric oxide synthase (NOS), may drive eNOS to produce superoxide instead of NO, which leads to the elevation of cellular ROS and vascular dysfunction in T2DM [43]. Furthermore, NO reacts with $O_2^{\bullet-}$ to produce ONOO⁻, which is implicated in diabetes by causing DNA damage/binding [44] and flow mediated dilation disruption [45]. Indeed, under normal physiologic conditions, there are many potential antioxidant defenses against mass-produced ROS, including enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidases (GPx), catalases (CAT), and non-enzymatic antioxidants (e.g., vitamins, metal ion chelators, glutathione). Nevertheless, once the balance between ROS and antioxidants is destroyed, oxidative stress will result.

ROS sources

Mitochondrial sources of ROS

ROS are produced mainly by the mitochondrial electron transport chain (ETC) during normal metabolic processes, defined as mitochondrial ROS (mtROS). The formation of mtROS arises from the transportation of one-electron of a redox donor to molecular oxygen (O_2) to produce $O_2^{\bullet-}$ [46]. A number of factors, including hypoxia, mitochondrial dysfunction and substrate availability, may elevate the production of mtROS by regulating the redox state of the ETC. Complex I (NADH-ubiquinone oxidoreductase) and complex III (ubiquinol-cytochrome C oxidoreductase) are pivotal reductases within ETC, and these two complexes represent major sites of superoxide generation. There are

also other mitochondrial ROS-producing sites, including monoamine oxidase [47], p66Shc [48], α -Glycerophosphate dehydrogenase [49], electron transfer flavoprotein (ETF), ETF quinone oxidoreductase (ETF dehydrogenase) [50], and aconitase [51].

Non-mitochondrial sources of ROS

While mitochondria are the major source of ROS, there are many non-mitochondrial sources of ROS, including, but not limited to, nicotinamide adenine nucleotide phosphate oxidase (NOX), eNOS, xanthine oxidase (XO), lipoxygenases, cyclooxygenases (COXs), monoamine oxidases, hemoxygenases (HO) and cytochrome P450 reductase (CYPOR) [52–55]. In particular, NOX and eNOS play significant roles in ROS generation relevant to the origin and development of diabetes and vascular diseases. NOX comprises 7 family members that produce ROS by catalyzing the electron transfer from nicotinamide adenine nucleotide phosphate (NADPH) to O_2 [56–59]. ROS generation induced by the elevated levels of NOX is associated with diabetes and its vascular complications. Knockdown of NOX1 and NOX4 decreases diabetes mellitus-accelerated atherosclerosis through diminution of ROS production [60]. eNOS represents the constitutive NOS form in many cell types, particularly endothelial cells, and catalyzes the generation of NO (which regulates vascular tone and normalizes vascular function) in physiologic conditions [61]. On the other hand, low tetrahydropterin/dihydropterin (BH4/BH2) ratios and elevated asymmetric dimethylarginine (ADMA) concentrations can result in eNOS uncoupling which increases the production of superoxide with diminished synthetic activity of NO [62]. The diminished bioavailability of NO leads to dysfunction of the endothelial progenitor cells (EPCs) which maintain blood vessel function by differentiating into mature endothelial cells and promoting the repair of the damaged endothelium [63]. Thus NO diminution plays a key role in diabetes related vascular complications, and numerous hypotheses suggest that supplementary NO donors (such as dietary nitrate and citrulline malate) are a potential means of alleviating the risk of diabetic vascular complications in people with diabetes.

Antioxidants

An antioxidant is a chemical substance responsible for regulating redox state by restraining and/or retarding the oxidation of other substrates including fat, oil, and food (Table 1) [64]. These beneficial molecules have high efficiency and defend against free radical-induced oxidative stress by three major mechanisms: (1) ROS scavenging agent: facilitating the diminution of ROS;

(2) Chelating agent: inhibiting the generation of free radicals by complexing metals; (3) Phenolic agent: preventing the propagation reaction by exchanging protons with free radicals [65]. The catalytic antioxidants (SOD, CAT and GPx) scavenge ROS by catalyzing the reduction of $O_2^{\cdot-}$, the breakdown of H_2O_2 or lipid hydroperoxides to H_2O and lipid alcohols [66–68]. Vitamin C can also act as a ROS scavenger by donating an electron to a substrate such as $O_2^{\cdot-}$ [69]. Besides ROS scavenging agents, chelating agents (such as proteolysis-induced peptides, carnosine and anserine) play a critical role in preventing the generation of ROS by complexing some metals (including Fe, Cu, and Mg) [70]. These trace elements promote the production of ROS such as $\cdot OH$ which is produced by Fe^{2+} -catalyzed the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow \cdot OH + OH^- + Fe^{3+}$). In addition to ROS scavengers and active iron chelators, vitamin E, a phenolic agent, can donate a hydrogen atom from the phenolic group located on the chromanol to lipid peroxide radicals in membranes and/or LDL [71]. The oxidized vitamin E is relatively stable and does not mediate further oxidative chain reaction. Furthermore, some other antioxidants, such as vitamin D, vitamin B₉, coenzyme Q10 (CoQ10), N-acetylcysteine (NAC), and lipoic acid (LA), modulate the levels of ROS indirectly [27,72].

Oxidative stress in diabetes mellitus

Sustained hyperglycemia leads to ROS overproduction by enhancing mitochondrial oxygen consumption, damaging mitochondrial function, or activating NOX that are evolutionarily conserved ROS-producing enzymes. The increased generation of ROS or a declined activity of endogenous antioxidants, or both, results in oxidative stress which is a potent culprit in diabetes mellitus by inducing β -cell dysfunctions and insulin resistance. In addition, oxidative stress is closely related with diabetic complications which are responsible for both the death and long-term disability of patients with diabetes.

Role of oxidative stress in β -cell dysfunction

The central feature of diabetes mellitus is impaired insulin secretion that is associated with overstimulation of β -cells by chronic hyperglycemia or free fatty acids (FFA) [73] (Fig. 2). Increasing evidence suggests that hyperglycemia and high FFA-induced ROS/RNS accumulation could more easily impair β -cell function due to the subnormal expression of antioxidants (SOD, CAT, GPx) in β -cells [74]. Chronic exposure of β -cells to oxidative stress inhibits insulin secretion by opening ATP-sensitive K^+ channels and suppressing calcium influx, which results from the ROS-induced overproduction of cyclin-dependent kinase inhibitor p21 [75]. In addition, excessive long-

Table 1 Antioxidants

Major antioxidants	Main functions	References
Enzymatic antioxidants		
SOD	Catalyzes $2\text{O}_2^{\cdot -} + 2\text{H}^+ \rightleftharpoons \text{O}_2 + \text{H}_2\text{O}_2$	[66]
CAT	Catalyzes $2\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{H}_2\text{O}$	[67]
GPx	Catalyzes the breakdown of H_2O_2 and lipid hydroperoxides to H_2O and lipid alcohols	[68]
Vitaminic antioxidants		
Vitamin C	Scavenges free radicals	[69]
Vitamin E	Scavenges lipid peroxide radicals in membranes	[71]
Vitamin D	Modulates the expression of antioxidants	[155]
Vitamin B ₉	Inhibits NOX4/Vav2/NLRP3 signaling	[156]
Other antioxidants		
GSH	Scavenges free radicals	[157]
CoQ10	Improves mitochondrial dysfunction	[158]
NAC	Reduces glutathione	[159]
LA	Cofactor for pyruvate dehydrogenase complex	[160]
Trace elements	Involves in redox cycling reactions	[27]

SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; NOX4, nicotinamide adenine nucleotide phosphate oxidase 4; NLRP3, nucleotide binding oligomerization domain-, leucine-rich repeat- and pyrin domain-containing 3; CoQ10, coenzyme Q10; NAC, N-acetylcysteine; LA, lipoic acid.

chain acyl CoA will be generated in the process of increased β -cell fatty acid metabolism which can keep β -cell ATP-sensitive K^+ channels open to suppress ATP generation and insulin secretion [76]. Long-term exposure of β -cells to elevated FFA drops the mitochondrial membrane potential and leads to uncoupled proteins-2 (UCP2) accumulation, which can also open β -cell ATP-sensitive K^+ channels to inhibit insulin production [77].

Oxidative stress may also decrease the transcriptional activity of insulin genes by reducing the nuclear accumulation of pancreas duodenal homeobox factor 1 (PDX-1, a key transcription factor responsible for maintaining β -cell function) [78]. Recent studies have demonstrated that oxidative stress-induced JNK activation and enhanced nuclear translocation of forkhead box protein O1 (FOXO1, a key driver of metabolic disease) can suppress the binding of PDX-1 to DNA [79,80]. It has been reported that ROS overproduction reduces insulin secretion by suppressing the expression of MaFA, a member of the fundamental leucine zipper family of transcription factors involved in the transcription of insulin genes [81]. By contrast, poor insulin secretion can be improved by inhibiting p38 MAPK-mediated MafA loss in *db/db* mice [82]. These results suggests that ROS may activate p38 MAPK to promote the degradation of MaFA and then have impact on β -cell function and insulin generation [83]. In addition, β -cells can also be damaged and even undergo apoptosis under chronic oxidative stress-mediated inflammation in which nuclear transcription factor NF- κ B is activated through IL-1R signaling to promote the expression of the Bcl-2 family proapoptotic members [84,85]. In summary, free radical overproduction may cause β -cell dysfunction and even apoptosis by regulating important

intracellular signaling pathways, such as p38 MAPK, JNK, and NF- κ B.

Role of oxidative stress in insulin resistance

When the levels of blood glucose are elevated, insulin will interact with insulin receptor in certain tissues (skeletal muscle, adipose tissue, and liver) and subsequently activate insulin signaling to promote glucose uptake and metabolism. Thus the interruption of insulin signaling is implicated in insulin resistance which plays a critical role in the occurrence and development of diabetes mellitus.

Under normal physiologic conditions, binding of insulin to the insulin receptor leads to tyrosine-phosphorylation of insulin receptor substrates (IRS). The tyrosine-phosphorylated IRS proteins then activate the phosphatidylinositol-3-kinase (PI3-kinase) p110 catalytic subunit by interacting with the p85/55/50 regulatory subunit of PI3-kinase [86]. The activated PI3-kinase can subsequently activate 3-phosphoinositide-dependent kinase (PDK1) and promote generation of phosphatidylinositol-3,4,5-triphosphate (PIP3) which causes the phosphorylation of Akt on Thr308 via the PDK1 kinase. The phosphorylated Akt can activate the AS160 (TBC1D4 which is a GTPase activating protein for Rab14) and TBC1D1 by phosphorylating their Rab-GTPase domain to promote GLUT4 translocation and glucose uptake.

Indeed, millimolar ROS concentrations play an important role in promoting signal transduction of insulin by an NADPH oxidase-dependent mechanism under physiologic conditions. Insulin stimulation elicits a short-term and low-dose ROS by mediating the generation of H_2O_2 . Moderate ROS, as the key messenger, modulates the enzymatic

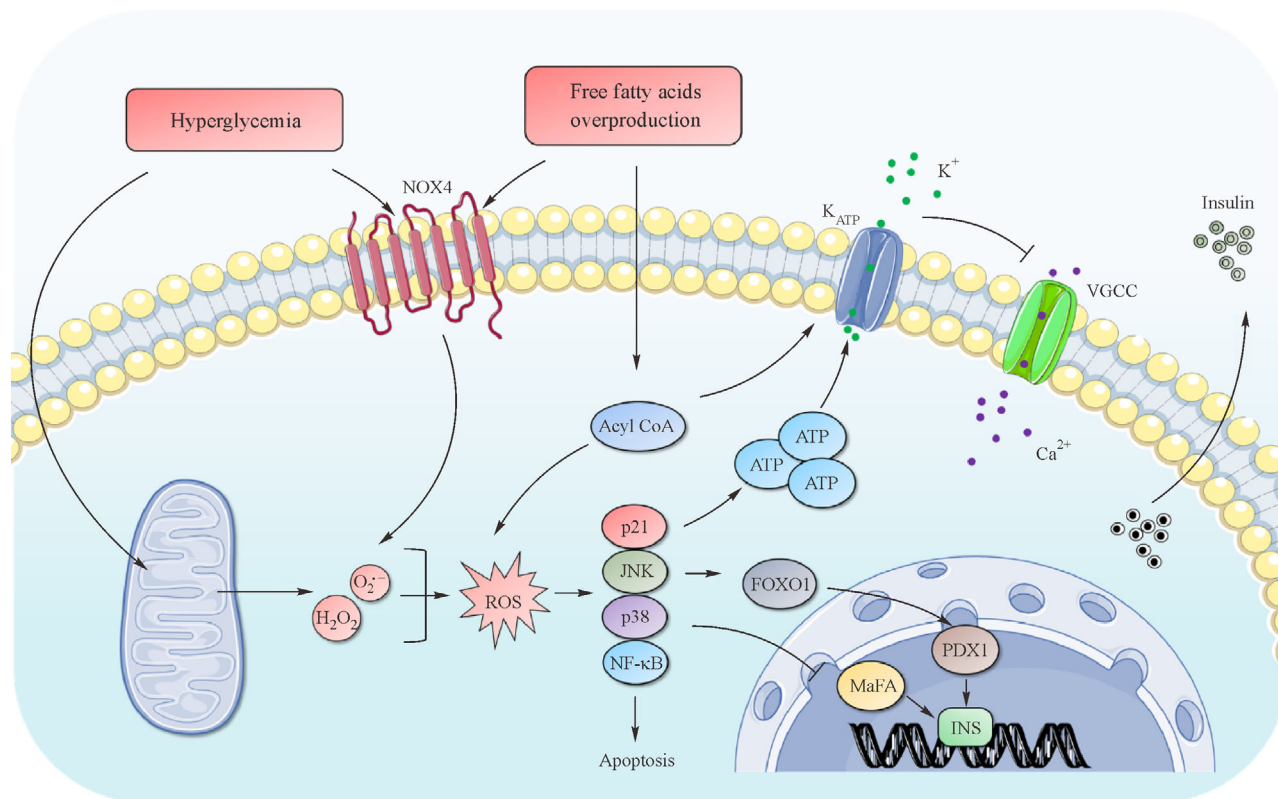


Fig. 2 Oxidative stress and pancreatic β -cell dysfunction. Oxidative stress mainly influences β -cell function from two perspectives: reducing insulin secretion and promoting β -cell apoptosis. On the one hand, ROS overproduction suppresses insulin production and secretion by opening ATP-sensitive K^+ channels and inhibiting insulin genes transcription. On the other hand, oxidative stress induces β -cell apoptosis by activating $p21$, JNK, $p38$ MAPK, and $NF-\kappa B$. Abbreviations: NOX4, nicotinamide adenine nucleotide phosphate oxidase; K_{ATP} , ATP-sensitive K^+ channels; VGCC, voltage-gated calcium channels; $p21$, a cyclin-dependent kinase inhibitor; JNK, c-jun N-terminal kinase; $p38$ MAPK, $p38$ AMP-activated protein kinase; $NF-\kappa B$, nuclear transcription factor κB ; $FOXO1$, forkhead box protein O 1; PDX1, pancreas duodenal homeobox factor 1; MaFA, musculoaponeurotic fibrosarcoma protein A; INS, insulin genes.

targets (including protein tyrosine phosphatases) and increases the basal tyrosine phosphorylation level of both the insulin receptor and its substrates [87]. However, hyperglycemia-mediated ROS overproduction and elevated FFA can lead to insulin resistance by impairing insulin signals (Fig. 3) and activate proinflammatory signaling proteins (mainly in adipose tissue). There are several serine-threonine kinase pathways activated by oxidative stress, such as $p38$ MAPK, JNK, and $IKK\beta/NF-\kappa B$. These kinases increase serine-phosphorylation of IRS and lead to IRS degradation that impairs insulin signaling pathways [88–90]. However, insulin resistance can be alleviated by inhibiting the activation of $p38$ MAPK, JNK1, and $IKK\beta$ [88,91,92]. All these findings are consistent with the connection between oxidative stress and insulin resistance. Other targets within insulin signaling pathways are also involved in insulin resistance due to redox imbalance. For example, ANG II (angiotensin II)-induced ROS accumulation leads to insulin resistance of L6 myotubes by preventing the tyrosine phosphorylation of Akt/GSK-3 [93]. Interestingly, recent evidence

indicated that elevated ROS promote the translocation of GLUT4 to lysosomes rather than sarcolemmal membrane by activating casein kinase-2 (CK2) which can suppress the trans-Golgi by enhancing the activity of the retromer complex [94].

Role of oxidative stress in diabetic vascular complications

Oxidative stress has been implicated in the pathogenesis and progression of diabetic vascular complications, including neuropathy, retinopathy, nephropathy, and cardiovascular disease [95]. Unlike other cells (skeletal muscle cells, adipocytes, and liver cells), the vascular endothelial cells show a lower-capability to decrease glucose uptake when extracellular glucose levels increase [96]. These result in endothelial intracellular hyperglycemia and subsequent damage by hyperglycemia-induced oxidative stress. Hyperglycemia-induced ROS overproduction is involved in vascular endothelial dysfunction by four major mechanisms (Fig. 4): an increase in intracellular

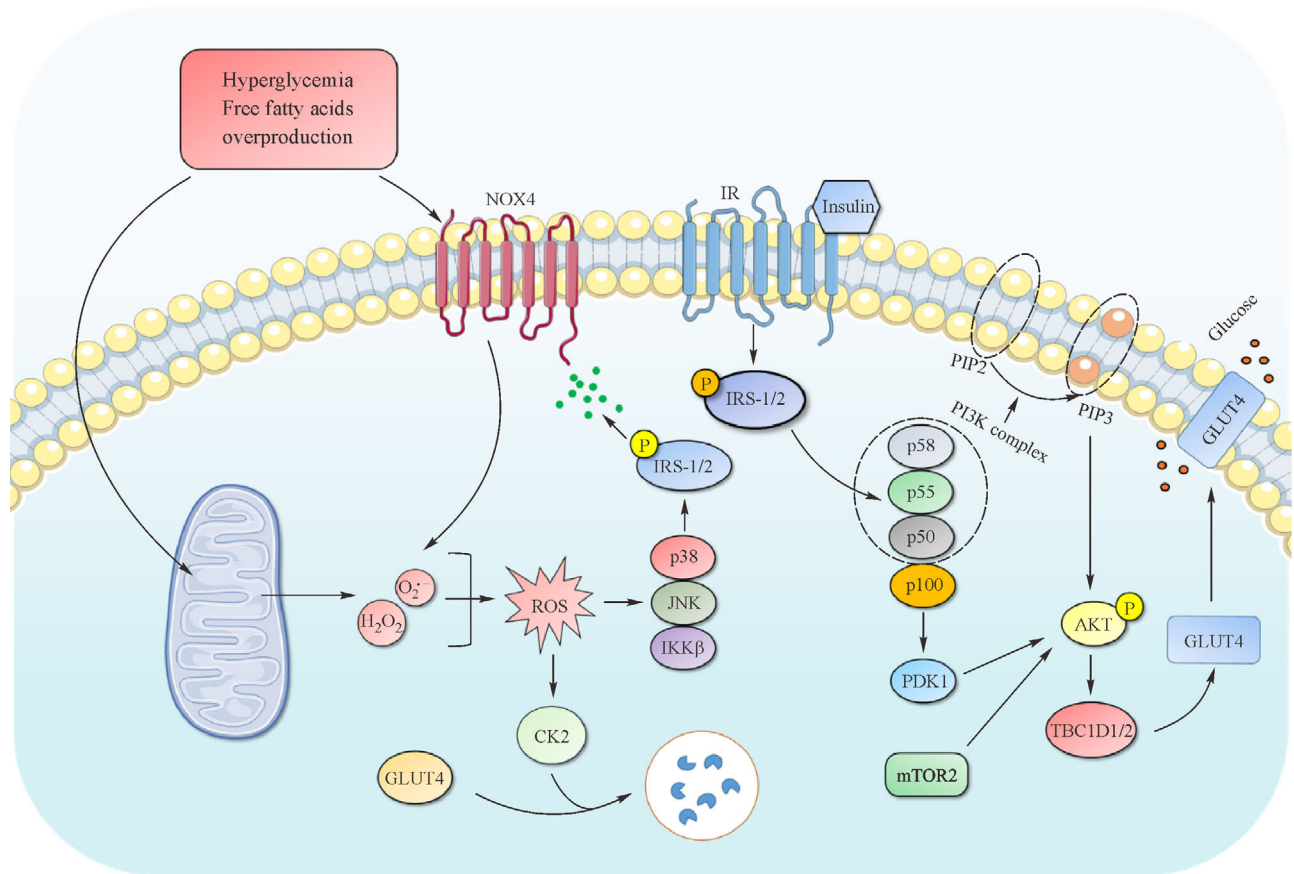


Fig. 3 Oxidative stress and insulin resistance in skeletal cells. Glucose traverses the membrane of muscle cells by a facilitative diffusion process which relies on the GLUT4 glucose transporter translocation from intracellular storage depots to the sarcolemmal membrane and T-tubules upon muscle contraction. The GLUT4 translocation is modulated by insulin through the activation of a complex cascade of signaling events. Under oxidative stress due to sustained hyperglycemia, elevated FFA inhibits glucose transportation by impairing insulin signals. ROS decreases insulin sensitivity by activating casein kinase-2 (CK2) which promotes the translocation of GLUT4 to lysosomes rather than the sarcolemmal membrane. Abbreviations: NOX, nicotinamide adenine nucleotide phosphate oxidase; IR, insulin receptor; IRS-1/2, insulin receptor substrates-1/2; H_2O_2 , hydrogen peroxide; $O_2^{\cdot-}$, superoxide; ROS, reactive oxygen species; JNK, c-jun N-terminal kinase; IKK β , I κ B kinase β ; CK2, casein kinase-2; GLUT4, glucose transporter 4; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-triphosphate; PDK1, 3-phosphoinositide-dependent kinase; mTOR2, mechanistic target of rapamycin 2; TBC1D1/2, Tre-2/BUB2/cdc 1 domain family 1/2.

advanced glycation and end products (AGEs) and their receptors; elevated glucose flux through the polyol pathway; activation of PKC; and enhanced activity of the hexosamine pathway.

Initially, intracellular hyperglycemia suppresses the activity of GAPDH which is a critical glycolytic enzyme. However, this inhibition can be alleviated by preventing mitochondrial superoxide overproduction with MnSOD [97]. Thus intracellular hyperglycemia may decrease GAPDH activity by mediating ROS overproduction. The low activity of GAPDH elevates the levels of upstream proteins (the glycolytic intermediates) leading to enhancement of these four major pathways [98].

Advanced glycation end products (AGEs) are generated by non-enzymatic glycosylation reaction in which amino

groups of proteins are glycated by the products of glucose and free fatty acid oxidation [99]. Hyperglycemia enhances this non-enzymatic reaction to elevate the generation of AGEs which promote structural change of some plasma proteins and then activate AGE receptors (RAGE). The binding of AGEs to RAGE in various cell types (e.g., endothelial cells, smooth muscle cells, macrophages, monocytes, and lymphocytes) contributes to NOX-induced ROS generation, leading to the activation of NF- κ B [100]. The activation of NF- κ B induces inflammation and thrombosis of vascular endothelial cells by further promoting the transcription of several genes, including endothelin-1 (ET-1, an endothelium-derived potent vasoconstrictor), vascular adhesion molecular-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), vascular

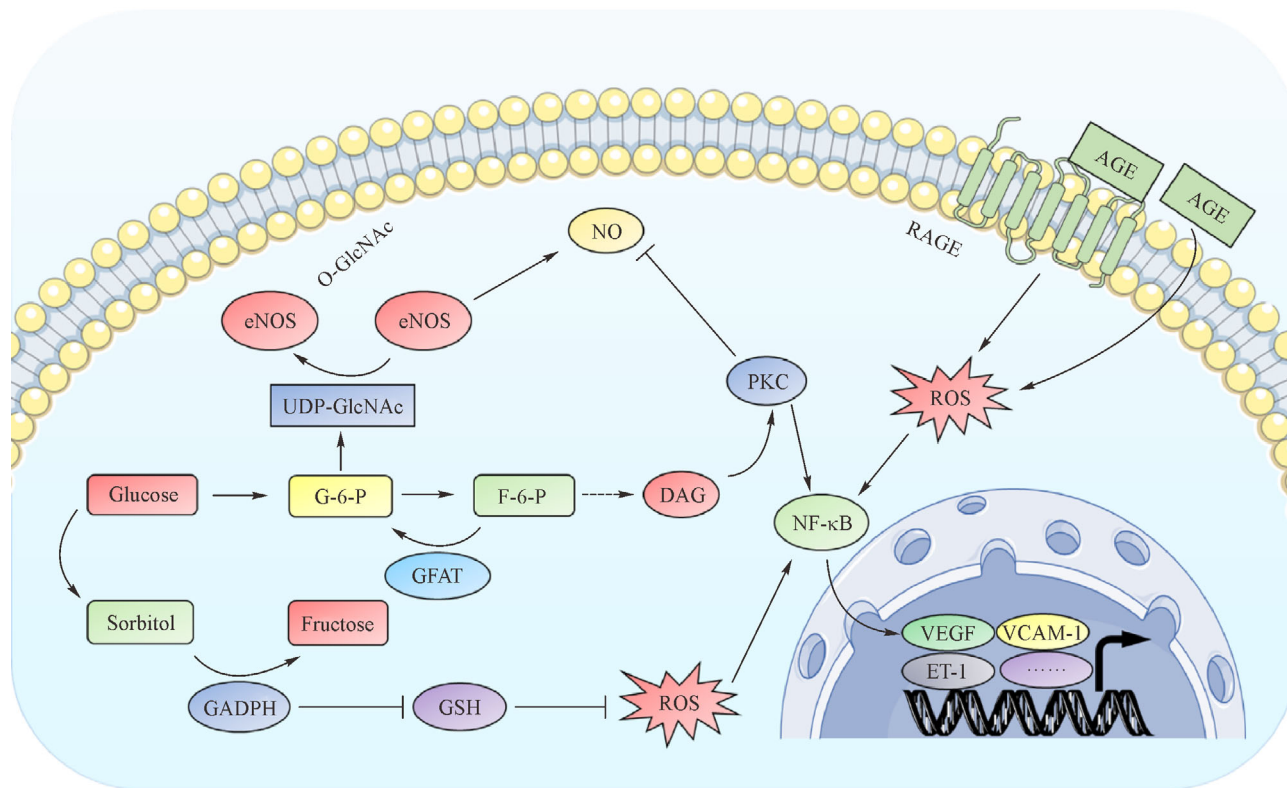


Fig. 4 Oxidative stress and vascular endothelial dysfunction. There are four major mechanisms associated with vascular endothelial cell dysfunction, including the PKC, AGEs/RAGE, polyol and hexosamine pathways. The PKC and hexosamine pathways diminish the generation of NO which is a critical regulatory factor to normalize vascular function. The polyol and AGEs/RAGE pathways elevate the levels of ROS in endothelia cells and then activate NF- κ B which induces the inflammation and thrombosis of vascular endothelia by enhancing several genes expression including VEGF, VCAM-1 and ET-1. Abbreviations: eNOS, endothelial nitric oxide synthase; O-GlcNAc, O-N-acetylglucosamine; NO, nitric oxide; PKC, protein kinase C; ROS, reactive oxygen species; AGE, advanced glycosylation end products; RAGE, receptor for advanced glycosylation end products; UDP-GlcNAc, uridine diphosphate N-acetylglucosamine; G-6-P, glucose 6 phosphate; F-6-P, fructose 6 phosphate; DAG, diacylglycerol; GFAT, glutamine fructose-6-phosphate aminotransferase; GADPH, D-glyceraldehyde-3-phosphate dehydrogenase; GSH, glutathione; NF- κ B, nuclear transcription factor κ B; VEGF, vascular endothelial growth factor; VCAM-1, vascular adhesion molecular-1; ET-1, endothelin-1.

endothelial growth factor (VEGF), tissue factor (TF), macrophage inflammatory protein-1 (MIP-1), thrombomodulin, IL-1, IL-6, and TNF- α [101]. In addition, high glucose decreases eNOS levels of vascular endothelia cells by inhibiting the transactivation of the transcription factor hypoxia-inducible factor-1 α (HIF-1 α), thus reducing the levels of NO, a critical regulatory factor for normalizing vascular function [102]. The decreased levels of NO thus perturb endothelial function and vascular homeostasis.

The polyol pathway is one of the most important processes in carbohydrate metabolism in which aldose reductase reduces glucose to sorbitol by NADPH [98]. The increased concentrations of intracellular glucose consume a large amount of NADPH which is the essential cofactor for constantly producing reduced glutathione (GSH). Decreased regeneration of GSH causes oxidative stress due to the overproduction of ROS because GSH plays a significant role in scavenging ROS. The elevated ROS play

detrimental roles in diabetic end-organ damage, particularly oxidative stress-mediated endothelial dysfunction. Many tissues (nerve, glomerulus, and vascular) express aldose reductase and can be damaged by diabetic hyperglycemia. In diabetic dogs, an aldose reductase inhibitor prevented diabetes-induced nerve conduction velocity defect [103]. This study indicated that the polyol pathway may play an important role in the pathogenesis of neuropathy.

PKC is a serine-threonine protein kinase consisting of 15 isoforms, which phosphorylate multiple target proteins to modulate cell growth, proliferation, senescence, and apoptosis. The activity of PKC can be regulated by several elements, such as Ca^{2+} ions, phosphatidylserine, and especially diacylglycerol (DAG) which can activate 9 isoforms of PKC [104–106]. In diabetic patients, hyperglycemia promotes the *de-novo* synthesis of DAG from the intermediate product of glucose metabolism, triose

phosphate. The increased generation of DAG leads to the excessive enhancement of PKC activation. The high-activity PKC induces the overproduction of ROS by inhibiting the glycolytic enzyme GAPDH, which in turn increases the availability of triose phosphate to generate DAG [107,108]. The activation of PKC leads to endothelial dysfunction, increases vascular permeability, and inhibits angiogenesis by many mechanisms, including activating JNK, extracellular regulating kinase 1/2 (ERK1/2) [109], and NF- κ B [110], and diminishing NO generation by suppressing insulin-stimulated expression of eNOS [111,112].

The hexosamine biosynthetic signaling pathway (HBP) aggravates hyperglycemia-induced diabetic complications by reducing NO production and promoting the possible transcription of some tissue growth factors (TGF- α and TGF- β 1). Initially, glutamine fructose-6-phosphate aminotransferase (GFAT), the rate-limiting enzyme of this pathway, catalyzes the conversion of fructose 6-phosphate to glucosamine 6-phosphate. Subsequently, glucosamine 6-phosphate is metabolized to uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), which is the primary substrate for post-translational modifications of several proteins including eNOS by generating O-GlcNAc at serine and threonine residues. Hyperglycemia and high

free fatty acid oxidation enhances HBP by increasing fructose 6-phosphate production. Enhanced HBP impairs endothelial function since O-GlcNAcylation restrains the phosphorylation of Akt/eNOS in endothelial cells and then decreases NO production [113]. Thus inhibition of HBP may be a potential therapeutic target for diabetic vascular complications.

Redox modification of proteins associated with diabetes

The physiologic and pathophysiological effects of ROS are modulated by reversible and irreversible redox modifications of proteins [114,115] (Fig. 5). Because of its chemical characteristics, ROS can modify several amino acids, including cysteine, methionine, and tyrosine. Among these amino acids, cysteine residues are the most readily modified in response to ROS because of a highly reactive thiol group [116]. Different ROS induce diverse thiol reactions, including S-glutathiolation, disulfide bonds [117], and S-nitrosylation [118]. Overproduction of ROS is implicated in the redox modification of cysteine residues within some proteins (IKK β , PKC, and Keap1) involved in the development and progression of diabetes and related complications.

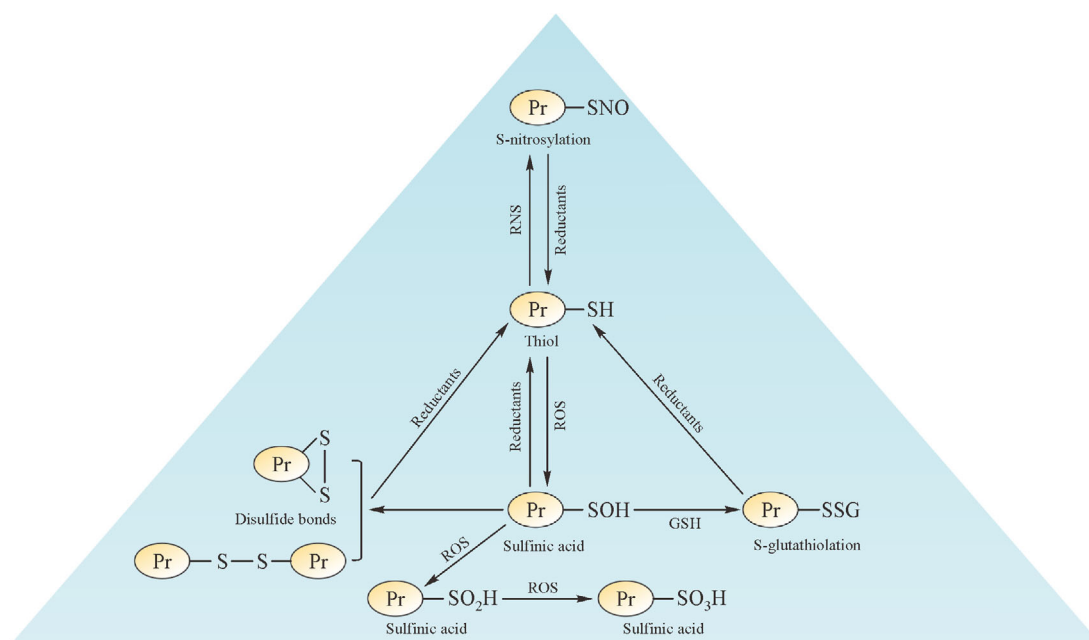


Fig. 5 Patterns of redox protein modification. The highly reactive thiol groups of proteins are easily oxidized to sulfenic acid (RSOH) by ROS, or are oxidized to S-nitrosylation in response to RNS. Sulfenic acid (RSOH) has the capacity to react with nearby thiols to form intramolecular or intermolecular disulfide bonds due to its highly reactive nature. Sulfenic acid (RSOH) can also react with GSH to generate S-glutathiolation. These redox proteins modifications are reversible and these reaction products can be restored into free thiols by cellular reductants. However, sulfenic acid (RSOH) can also be further oxidized to irreversible products (including RSO₂H and RSO₃H). Abbreviations: ROS, reactive oxygen species; RNS, reactive nitrogen species; GSH, glutathione.

Redox modification in the regulation of IKK β /NF- κ B

Oxidative stress activates the IKK β /NF- κ B pathway which may cause pancreatic β -cell dysfunction and inflammation associated with diabetic vascular complications. Without NF- κ B-activating stimuli, NF- κ B are sequestered in the cytoplasm as a latent form by IKK β . When IKK β is degraded due to tissue factors such as TNF- α , untethered NF- κ B is translocated into the nucleus and activates the transcription of genes relevant for inflammation and cell survival [119,120]. Recently, anecdotal evidence suggests that ROS promotes the degradation of IKK β via redox modification of cysteine. For example, H₂O₂ has been shown to induce IKK β cysteine oxidation and suppress the activity of IKK β , but the cysteine sites involved remain unclear [121]. Kapahi and coworkers found that arsenite oxidized Cys179 of IKK β and inhibited IKK β activity, suggesting Cys179 may be a potential redox sensitive site [122].

Redox modification of PKC

As discussed above, the activation of PKC can induce the overproduction of ROS, and in turn ROS can oxidize cysteine residues of specific PKC isoforms leading to activation of PKC and downstream signaling associated with diabetic complications [123]. Recent studies found that the N-terminal regulatory domain of PKC contains zinc thiolates whose oxidative modification can enhance PKC activity by the absence of Ca²⁺ and phospholipid auto-inhibition effects [124]. On the other hand, further studies suggest that chemopreventive antioxidants (such as vitamin E) interact with cysteine residues within the C-terminal catalytic domain and then inhibit cellular PKC activity [124,125]. These results suggest redox modification of PKC may be of pivotal importance in the pathogenesis and development of diabetic complications, and targeting PKC redox modification holds great potential for the treatment of diabetes mellitus.

Redox modification in the modulation of the Nrf2/Keap1/ARE pathway

The Nrf2/Keap1/ARE pathway plays an important role in the regulation of cellular redox homeostasis. Nrf2 (nuclear factor E2-related factor 2) represents a master transcription factor, which regulates the antioxidant response element (ARE) sequence and promotes the expression of antioxidants including HO-1, NADPH dehydrogenase [quinone] 1 (NQO1), CAT, and SOD. As a component of the cullin-3 based ubiquitin E3, Keap 1 (Kelch-like ECH-associated protein 1) inhibits the transcriptional activity of Nrf2 by inducing Nrf2 ubiquitination and subsequent proteosomal degradation under homeostatic conditions. Under stress

conditions, the sensor cysteine residues of Keap1 are modified and the ubiquitination process of Nrf2 is suppressed. It has been reported that the Keap1 possesses five cysteine sensors (Cys151, Cys226, Cys273, Cys288, and Cys613) whose oxidative modifications weaken the interaction between Keap1 and cullin-3, probably through intramolecular or intermolecular disulfide bridges formation [115,126–129]. Liberated Nrf2 binds to the ARE and induces the transcription of its target genes such as HO-1, NQO-1, CAT, and SOD to protect cells from various stresses (ROS/RNS). Therefore, the Nrf2/Keap1/ARE pathway has emerged as a potential therapeutic target for many diseases associated with oxidative stress, including diabetes. Studies by Uruno *et al.* in Keap1 knockdown mice with diabetes suggest that Nrf2 overexpression can ameliorate insulin resistance [130]. In addition, a plethora of evidence indicates that many natural-occurring substance can activate Nrf2 by modification of Keap1 cysteine residues to alleviate diabetes and prevent diabetic end-organ damage. For example, sulforaphane (SFN) found in cruciferous vegetables has renoprotection effects in streptozotocin (STZ)-induced diabetic mice [131]. Another example is curcumin (CUR) in turmeric which can also induce modification of Keap1 cysteine residues and reduces the number of prediabetic patients who may develop T2DM [132]. Taken together, these results suggest that targeting modification of Keap1 cysteine residues may be a promising therapeutic strategy for diabetes by modulating the Nrf2/Keap1/ARE pathway.

Therapeutic approaches for diabetes: controlling oxidative stress

Given oxidative stress and diabetes go hand in hand, a number of tentative diabetic therapeutic strategies targeting oxidative stress have recently been attempted using antioxidant supplementation, such as enzymatic antioxidants-like mimics (SOD/CAT/GPx mimetics), vitamins (A, C, E), β -carotene, flavonoids, selenium, zinc, NAC, and CoQ10. However, such management of antioxidants has not shown any benefit for diabetes and diabetic complications in human clinical trials [133,134]. The poor performance of antioxidants supplements may result from their poor solubility, permeability, stability, and specificity [11]. For example, dietary CoQ10 is poorly bioavailable as it has poor solubility because of its hydrophobicity and large molecular weight and rarely reaches the mitochondria [135]. However, when CoQ10 is formulated with a novel carrier, delocalized lipophilic cation (DLC), it can be effectively delivered to mitochondria and has a higher efficiency [136]. Thus novel delivery systems for antioxidants are essential to enhance the effects of these supplements in the treatment of diabetes. Furthermore,

lifestyle management and precise pharmacological interventions alleviate diabetes and related complications by targeting oxidative stress. We next discuss several precise therapeutic approaches targeting redox modification. Such therapies will change our concepts of general antidiabetic treatment (Table 2).

Exercise and dietary control

T2DM is most common in the aged population, while younger people may become predisposed to the disease due to obesity, physical inactivity, and poor diet (International Diabetes Federation, 2017). Thus a healthy lifestyle is an effective mode of intervention to prevent T2DM. Regular aerobic and resistance exercise have been shown to improve the metabolic disturbance of diabetes and its complications by inducing glycemic control, insulin sensitivity, body composition and controlling blood pressure and lipid profile [137–139]. Moe *et al.* found that inactive people with diabetes had three times the risk of cardiovascular death compared with those without diabetes, but the increased risk was diminished by increasing physical exercise [140]. Indeed, the activity of some antioxidants, such as MnSOD and GPx, are rapidly increased through short-term endurance exercise [141]. In addition, depending on the status of diabetes individuals including age, comorbidities and other specific conditions, dietary intervention by avoiding transfat, saturated fat, cholesterol while increasing the intake of fiber, ω -3 fatty acids and plant pigments (resveratrol, cyanidins, curcumin, theaflavins, quercetin) can be a beneficial approach for preventing the progression of diabetes [142].

Novel antioxidant delivery systems

Some dietary antioxidants, such as catalase, vitamin C, and lipoic acid, have poor solubility, permeability, and stability in conventional delivery systems, which may result in disappointing outcomes in clinical trials [11]. Recently, a number of novel drug delivery systems (NDDS) have been reported to improve the solubility, permeability, and stability of antioxidants including microparticles, nanoparticles, and liposomes. Microparticulate drug delivery system can promote the entry of antioxidants with poor membrane permeability (such as SOD) into cells. Polyketals are a new family of acid-degradable polymers with ketal linkages in their backbones that are being developed for intracellular drug delivery. Poly (cyclohexane-1,4-diyl acetone dimethylene ketal) (PCADK) which degrades into acetone, a compound recognized as safe by the FDA and 1,4-cyclohexanedimethanol which has an excellent toxicity profile have been used to encapsulate SOD and form SOD-PCADK microparticles. Using a cell-based assay (TIB-186 macrophages), they found that SOD-PCADK microparticles caused a 60% reduction in superoxide production whereas free SOD had little effect [143]. NDDS-coated antioxidants boost antioxidative efficiency not only in cell-based experiments, but also in animals. Nanoparticles are another NDDS which can increase the bioavailability of antioxidants. For instance, biodegradable curcumin encapsulated nanoparticles were found to delay the progression of cataracts in a diabetic rat model [144]. Another study suggests that curcumin also has higher antioxidative capacity when encapsulated into liposomes (artificial lipid bilayer vesicles) [145], although their shelf

Table 2 Therapeutic antioxidative strategies for diabetes

Antioxidative strategies	Main functions	References
Lifestyle interventions		
Exercise	Increases muscle mitochondrial oxidative capacity and enhances NO bioavailability	[161]
Dietary	Decreases uptake of free fatty acids	[142]
NDDS		
Microparticle	Promotes the entry of antioxidants with poor membrane permeability	[143]
Nanoparticle	Increases the bioavailability of antioxidants	[144]
Liposome	Improves antioxidative capacity of antioxidants	[145]
Agents targeting ROS sources		
MitoQ-TPP	Prevents mitochondrial oxidative damage	[162]
TEMPOL	Prevents mitochondrial oxidative damage and improves tissue oxygenation	[163]
GKT137831	Inhibits the activation of caspase-3 and cell death resulted from high glucose	[148]
Agents targeting redox modification		
Bardoxolone methyl	Regulates the Nrf2/Keap1/ARE pathway through Keap1 post-translational modification	[150]
tBHQ	Regulates the Nrf2/Keap1/ARE pathway through Keap1 post-translational modification	[151]
Selenocompounds	Modifies PKC C-terminal catalytic domain and inhibits cellular PKC activity	[152]

NO, nitric oxide; NDDS, novel drug delivery systems; ROS, reactive oxygen species; Nrf2, nuclear factor E2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; ARE, antioxidant response element; tBHQ, tert-butylhydro-quinone; PKC, protein kinase C.

life is poor [118]. Taken together, NDDS have great potential for enhancing the effects of antioxidants in the treatment of diabetes. NDDS can also deliver antioxidants to the designated intracellular position, which may provide novel insight into non-selectivity of antioxidants. However, controlling antioxidant intake may be another major challenge in limiting antioxidant access to clinical applications.

Drugs targeting ROS sources

Diminishing the generation of ROS is the fundamental strategy to alleviate oxidative stress. A large number of clinical trials targeting ROS sources are currently in progress for diabetes and diabetic complications. MitoQ-TPP and TEMPOL are two mitochondrial-targeted antioxidants which diminish oxidative stress and blood pressure to alleviate vascular prognosis in patients with diabetes [146,147]. Furthermore, suppressing non-mitochondrial sources, particularly using NOX inhibitors, has also emerged as a potential treatment for diabetic complications. Several NOX inhibitors have been reported, including GKT137831, GKT136901, APX-115, and VAS2870. To date only GKT137831 has been used in clinical trials to alleviate diabetic nephropathy. However, phase II trials were disappointing due to failure to reduce albuminuria. *In vitro*, GKT137831 inhibited the activation of caspase-3 and cell death resulted from high glucose in cultured human retinal cells [148]. A possible mechanism is that GKT137831 can competitively inhibit NOX due to its similar structure [149]. Thus GKT137831 has potential as a therapeutic drug for diabetic retinopathy by reducing NOX-induced ROS production.

Promising drugs targeting redox modification

The redox modification of some proteins (IKK β , PKC, and Keap1) involved in diabetes plays a critical role in the pathogenesis of diabetes and related complications. Inhibiting or changing the redox modification of proteins may be a promising therapeutic method for diabetes and diabetic end-organ damage. It has been reported that bardoxolone methyl (CDDO-Me/RTA 402), a synthetic derivative of oleanolic acid, has shown potent efficacy in a short-term clinical trial in patients with T2DM by modifying the cysteine residues of Keap1 [150]. This study suggest that it is feasible to alleviate diabetes by regulating the Nrf2/Keap1/ARE pathway by manipulating Keap1 post-translational modification. Consistent with this idea, Zhong *et al.* found that tert-butylhydro-quinone (tBHQ), a synthetic preservative, inhibits the development of diabetic retinopathy by modification of the Keap1 cysteine residues [151]. In addition to Keap1 post-translational modification, PKC redox modification is

also associated with diabetic complications. Various chemopreventive antioxidants (selenocompounds, curcumin, and vitamin E) can modify the reactive cysteines within the PKC C-terminal catalytic domain and then inhibit cellular PKC activity [152]. Some studies suggest that selenocompounds can induce the inactivation of PKC to prevent tumor progression [153,154], although to date there is no report about the relationship between antioxidant-induced post-translational modification of PKC and diabetic complications. Nevertheless, regulation of PKC redox modification could have potential in the treatment of diabetic complications.

Conclusions and perspectives

Diabetes is characterized by hyperglycemia which leads to the overproduction of free radicals (ROS/RNS) and further increases oxidative stress due to the imbalance between ROS/RNS and antioxidants. In turn, multiple studies have suggested that oxidative stress plays a major role in the progression of diabetes involved with pancreatic β -cell dysfunction, insulin resistance, and diabetic complications. Thus, the therapeutic strategy of targeting oxidative stress has been shown to have great potential for the treatment of diabetes and diabetic complications. Although antidiabetic attempts with antioxidants, such as vitamin C/E supplementation and SOD-like mimics, these therapeutic approaches have been unsatisfactory possibly due to poor solubility, permeability, and stability. It is therefore necessary to develop novel delivery systems to improve the efficiency of antioxidants. In addition, precise therapeutic intervention is essential to inhibit ROS generation from mitochondria or non-mitochondrial sources. Recent studies suggest that the redox modification of some critical proteins (such as IKK β , PKC, and Keap1) are implicated in the pathogenesis and progression of diabetes and diabetic end-organ damage. Therapeutic strategies targeting post-translational modification of these proteins have shown a perspective potential in the treatment of diabetes. Controlling oxidative stress clearly has potential in the treatment of diabetes. However, it is necessary to develop novel antioxidant delivery approaches and precise drug targeting of ROS sources and redox modifications to prevent and retard the pathogenesis and progression of diabetes and diabetic complications.

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Compliance with ethics guidelines

Pengju Zhang, Tao Li, Xingyun Wu, Edouard C. Nice, Canhua Huang, and Yuanyuan Zhang declare no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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