Published in final edited form as:
Curr Med Chem. 2005 ; 12(3): 267–275.

Role of Nitrosative Stress and Peroxynitrite in the Pathogenesis of Diabetic Complications. Emerging New Therapeutical Strategies

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Abstract

Macro- and microvascular disease are the most common causes of morbidity and mortality in patients with diabetes mellitus. Diabetic cardiovascular dysfunction represents a problem of great clinical importance underlying the development of various severe complications including retinopathy, nephropathy, neuropathy and increase the risk of stroke, hypertension and myocardial infarction. Hyperglycemic episodes, which complicate even well-controlled cases of diabetes, are closely associated with increased oxidative and nitrosative stress, which can trigger the development of diabetic complications. Hyperglycemia stimulates the production of advanced glycosylated end products, activates protein kinase C, and enhances the polyol pathway leading to increased superoxide anion formation. Superoxide anion interacts with nitric oxide, forming the potent cytotoxin peroxynitrite, which attacks various biomolecules in the vascular endothelium, vascular smooth muscle and myocardium, leading to cardiovascular dysfunction. The pathogenetic role of nitrosative stress and peroxynitrite, and downstream mechanisms including poly(ADP-ribose) polymerase (PARP) activation, is not limited to the diabetes-induced cardiovascular dysfunction, but also contributes to the development and progression of diabetic nephropathy, retinopathy and neuropathy. Accordingly, neutralization of peroxynitrite or pharmacological inhibition of PARP is a promising new approach in the therapy and prevention of diabetic complications. This review focuses on the role of nitrosative stress and downstream mechanisms including activation of PARP in diabetic complications and on novel emerging therapeutical strategies offered by neutralization of peroxynitrite and inhibition of PARP.

Keywords

peroxynitrite; nitric oxide; superoxide; nitrotyrosine; diabetes; vascular; cardiomyopathy; nephropathy; neuropathy; retinopathy

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INTRODUCTION

Diabetic state is associated with increased oxidative stress, which plays an important role in the development of diabetic complications. Hyperglycemia stimulates the production of advanced glycosylated end products, activates protein kinase C, and enhances the polyol pathway leading to increased superoxide anion formation [1–3]. Superoxide anion interacts with nitric oxide, which is produced, physiologically, by constitutive sources, such as the endothelial isoform of nitric oxide synthase (eNOS). This process leads to the formation of the strong oxidant peroxynitrite, which attacks various biomolecules leading to cellular dysfunction *via* multiple mechanisms (Table 1) [1,2,4–47]. One of these pathways involves DNA strand breakage and activation of the nuclear enzyme poly(ADP-ribose) polymerase, which has been covered by separate overviews [6,48,49]. The present review summarizes the accumulating experimental and clinical evidence implicating the pathogenetic role of increased nitrosative stress, peroxynitrite formation in the development of diabetic complications (Table 2). Although peroxynitrite generation also plays a role in the pathogenesis of islet-cell destruction [18,30,50], this is a separate area which is not the main focus of the present review.

THE ROLE OF OXIDATIVE AND NITROSATIVE STRESS IN THE PATHOGENESIS OF DIABETES-INDUCED VASCULAR DYSFUNCTION

Various neurohumoral mediators and mechanical forces acting upon the innermost layer of blood vessels, the endothelium, are involved in the regulation of the vascular tone. A main pathway of vasoregulation involves the activation of the eNOS resulting in NO production [51]. Endothelium-dependent vasodilatation is frequently used as a reproducible and accessible parameter to probe endothelial function in various pathophysiological conditions. It is well established that endothelial dysfunction, in many diseases, precedes and predicts as well as predisposes for the subsequent, more severe vascular alterations. Endothelial dysfunction has been documented in various forms of diabetes, and even in pre-diabetic individuals [3,17,21, 52–57]. The pathogenesis of this endothelial dysfunction involves many components including increased polyol pathway flux, altered cellular redox state, increasedformation of diacylglycerol and the subsequent activation of specific protein kinase C isoforms, and accelerated nonenzymatic formation of advanced glycation end products [58–63]. Many of these pathways, in concert, trigger the production of oxygen- and nitrogen-derived oxidants and free radicals, such as superoxide anion and peroxynitrite, which play a significant role in the pathogenesis of diabetes-associated endothelial dysfunction [59–61,64]. The cellular sources of reactive oxygen species such as superoxide anion are multiple and include advanced glycation end products, NAD(P)H oxidases, the mitochondrial respiratory chain, xanthine oxidase, the arachidonic acid cascade (lipoxygenase and cycloxygenase), and microsomal enzymes [1,59].

Superoxide anion may quench NO, thereby reducing the efficacy of a potent endothelium-derived vasodilator system that participates in the homeostatic regulation of the vasculature, and evidence suggests that during hyperglycemia, reduced NO availability exists [65]. Hyperglycemia-induced superoxide generation contributes to the increased expression of NAD (P)H oxidase, which in turn generate more superoxide anion. Hyperglycemia also favors, through the activation of NF-κB an increased expression of iNOS, which may increase the generation of NO [56,66].

Superoxide anion interacts with nitric oxide, forming the strong cytotoxin peroxynitrite ($ONOO^-$), which attacks various biomolecules, leading — among other processes —to the production of a modified amino acid, nitrotyrosine [67]. Although nitrotyrosine was initially considered a specific marker of peroxynitrite generation, other pathways can also induce tyrosine nitration. Thus, nitrotyrosine is now generally considered a collective index of reactive

nitrogen species, rather than a specific indicator of peroxynitrite formation [68,69]. The possibility that diabetes is associated with increased nitrosative stress is supported by the recent detection of increased nitrotyrosine plasma levels in type 2 diabetic patients [8] and iNOS-dependent peroxynitrite production in diabetic platelets [15]. Nitrotyrosine formation is detected in the artery wall of monkeys during hyperglycemia [70] and in diabetic patients during an increase of postprandial hyperglycemia [10,11]. In a recent study we have demonstrated increased nitrotyrosine immunoreactivity in microvasculature of type 2 diabetic patients [17]. In the same study significant correlations were observed between nitrotyrosine immunostaining intensity and fasting blood glucose, HbA1c, intracellular adhesion molecule (ICAM), and vascular cellular adhesion molecule (VCAM).

The toxic actions of nitrotyrosine in the cardiovascular system are also highlighted by the evidence showing that there is increased apoptosis of endothelial cells, myocytes and fibroblasts in heart biopsies from diabetic patients [25], in hearts from streptozotocin-induced diabetic rats [26], and in working hearts from rats during hyperglycemia [28]. Importantly, the degree of cell death and/or dysfunction shows a correlation with levels of nitrotyrosine found in those cells. There is also evidence that nitrotyrosine can be directly harmful to endothelial cells [71]. In addition, high glucose-induced oxidative and nitrosative stress pathologically alters prostanoid profile in human endothelial cells [19,21].

Recent evidence indicates that there may be several phases to the pathogenesis of the endothelial injury induced by high glucose: the short-term effect appear to depend on a combined oxidative and nitrosative stress with peroxynitrite formation, whereas the long-term effect is related to reactive oxygen species generation; in both cases, protein kinase C ultimately mediates the vascular permeability changes [22].

Angiotensin II is a known factor in the pathogenesis of diabetic complications, perhaps most importantly, in nephropathy, cardiomyopathy and retinopathy. Recent studies indicate that the protective effects of angiotensin converting enzyme inhibitors or angiotensin receptor antagonists may go beyond the blood pressure lowering effects of these agents [72–74]. Furthermore, ACE inhibition *in vivo* reduces the apparent formation of peroxynitrite [35]. In this context it is noteworthy that angiotensin II can induce direct, pro-oxidative effects on the vascular endothelium. These effects are, at least in part, mediated by intraendothelial reactive species formation *via* a new family of NAD(P)H oxidase subunits, known as the non-phagocytic NAD(P)H oxidase proteins. Reactive oxidant species produced following angiotensin II-mediated stimulation of NAD(P)H oxidases can exert direct oxidative effects, but can also signal through pathways such as mitogen-activated protein kinases, tyrosine kinases and transcription factors, and lead to events such as inflammation, hypertrophy, remodeling and angiogenesis [54]. Recent work demonstrates that angiotensin II can also induce intraendothelial peroxynitrite formation [6,75–77], as well as PARP activation [76, 77].

THE ROLE OF OXIDATIVE AND NITROSATIVE STRESS IN THE PATHOGENESIS OF DIABETIC CARDIOMYOPATHY

Diabetic cardiomyopathy is characterized by complex changes in the mechanical, biochemical, structural, and electrical properties of the heart, which may be responsible for the development of an early diastolic dysfunction and increased incidence of cardiac arrhythmias in diabetic patients. The mechanism of diastolic dysfunction remains unknown but it does not appear to be due to changes in blood pressure, microvascular complications or elevated circulating glycated hemoglobin levels [78–82]. There is circumstantial clinical and experimental evidence suggesting that increased sympathetic activity, activated cardiac renin-angiotensin system, myocardial ischemia/functional hypoxia, and elevated circulating levels of glucose

result in oxidative and nitrosative stress in cardiovascular system of diabetic animals and humans. Oxidative stress associated with an impaired antioxidant defense status may play a critical role in subcellular remodeling, calcium-handling abnormalities, and subsequent diabetic cardiomyopathy [26,82,83].

Oxidative and nitrosative damage may be critical in the early onset of diabetic cardiomyopathy [18,25,26,28]. Even in simple model systems, e.g. placement of beating myocytes into culture medium containing elevated glucose, the pathophysiological alterations can be attenuated by antioxidants, NOS inhibitors, as well as by peroxynitrite neutralizing agents [84]. Consistently with the *in vivo* importance of this latter pathomechanism, significant nitrotyrosine formation was reported in cardiac myocytes from myocardial biopsy samples obtained from diabetic and diabetic hypertensive patients [25] and in a mouse model of STZ-induced diabetes [26]. Perfusion of isolated hearts with high glucose caused a significant upregulation of iNOS, increased the coronary perfusion pressure and both NO and superoxide generation, a condition favoring the production of peroxynitrite, accompanied by the formation of nitrotyrosine and cardiac cell apoptosis [28]. Fig. (1) shows increased NT formation in STZ-induced diabetic rat tissues.

PEROXYNITRITE NEUTRALIZATION IMPROVES CARDIAC AND VASCULAR DYSFUNCTION IN DIABETES

As mentioned above there is circumstantial evidence that nitrosative stress and peroxynitrite formation importantly contribute to the pathogenesis of diabetic cardiomyopathy both in animals and humans. We have tested a novel metalloporphyrin peroxynitrite decomposition catalyst, FP15, in murine models of diabetic cardiovascular complications [18]. We hypothesized that neutralization of peroxynitrite with FP15 would ameliorate the development of cardiovascular dysfunction in a streptozotocin-induced murine model of diabetes. In order to ensure that the animals received the FP15 treatment at a time when islet cell destruction was already complete and hyperglycemia has stabilized the treatment was initiated six week after the injection of streptozotocin. Although FP15 did not affect blood glucose levels, it provided a marked protection against the loss of endothelium-dependent relaxant ability of the blood vessels (Fig. 2A) and improved the depression of both diastolic (Fig. 2B) and systolic function of the heart [18]. The mechanism by which FP15 protects diabetic hearts from dysfunction may involve protection against vascular and myocardial tyrosine nitration, PARP activation, lipid peroxidation, and multiple other mechanisms, as all these mechanisms have previously been linked to diabetic cardiomyopathy as well as to peroxynitrite-induced cardiac injury. Additional mechanisms of peroxynitrite-mediated diabetic cardiac dysfunction may include inhibition of myofibrillar creatine kinase [85] and of succinyl-CoA:3-oxoacid CoAtransferase [27] or activation of metalloproteinases [45,86].

There are many pathophysiological conditions of the heart that are associated with peroxynitrite formation, including acute myocardial infarction, chronic ischemic heart failure, doxorubicin-induced and diabetic cardiomyopathy [86–91]. It appears that peroxynitrite decomposition catalysts improve cardiac function and overall outcome in these models. For instance, FP15 reduced myocardial necrosis in our current rat model of acute myocardial infarction [86] as well as in a recent porcine study [87]. Furthermore, FP15 significantly improved cardiac function in a doxorubicin-induced model of heart failure [86]. These observations-coupled with the recently reported protective effect of FP15 against diabetic cardiomyopathy-support the concept that peroxynitrite is a major mediator of myocardial injury in various pathophysiological conditions, and its effective neutralization can be of significant therapeutic benefit.

THE ROLE OF OXIDATIVE AND NITROSATIVE STRESS IN THE PATHOGENESIS OF DIABETIC RETINOPATHY, NEPHROPATHY AND EUROPATHY

Recent studies have suggested that increased oxidative and nitrosative stress is involved in the pathogenesis of diabetic microvascular injury in retinopathy nephropathy and neuropathy [33–46,92,93] (Table 2, Fig. (1)).

Retinal endothelial cells maintained in high glucose had significant increased eNOS expression and activity as well as increased formation of superoxide anion and nitrotyrosine [40,41]. Each of these alterations was blocked by the NOS inhibitor, L-NAME, or the peroxynitrite scavenger, uric acid. Consistently with these observation there is increased oxidative and nitrosative stress in retinas of diabetic animals, which is attenuated by antioxidant treatment [39–41]. In addition increased peroxynitrite-mediated VEGF and urokinase plasminogen activator receptor expression was demonstrated and proposed to be responsible for the breakdown of the blood-retina barrier in diabetic animals [40,41].

Increased oxidative stress and nitrotyrosine formation have also been demonstrated both in kidneys of diabetic animals [34–36] and in biopsies from patients with diabetic nephropathy [33] suggesting pathogenetic role in the development of this complication.

Although hyperglycemia has been proven to cause peripheral nerve dysfunction in patients with diabetes, the biochemical mechanisms for this effect are poorly understood [94]. Recent studies in experimental animals have indicated that hyperglycemia stimulates the production of nitric oxide, which reacts with superoxide anion to form peroxynitrite, which is damaging the endothelium and perineurium [42–44,46,93]. In a recent murine study, sciatic motor nerve conduction velocity and hind-limb digital sensory conduction velocity were reduced in diabetic mice versus controls, and both indices were normalized by FP15, a peroxynitrite decomposition catalyst compound [95], which also ameliorated the accumulation of poly(ADP-ribose) accumulation in diabetic nerves [95].

It is noteworthy that in preclinical studies, administration of the aldose reductase inhibitors sorbinil or fidarestat to diabetic rats not only corrected diabetes-induced depletion of glutathione and ascorbate, downregulation of SOD activity and accumulation of lipid peroxidation products in the peripheral nerve, superoxide formation in *vasa nervorum* and of diabetes-associated retinal oxidative and nitrosative stress, but also inhibited poly(ADP-ribose) accumulation (a marker of PARP activation) in diabetic nerve and retina [96].

PEROXYNITRITE-POLY(ADP-RIBOSE) POLYMERASE CONNECTION IN THE PATHOGENESIS OF DIABETIC COMPLICATIONS

Peroxynitrite also damages DNA and thus triggers the activation of DNA repair systems. A DNA nick sensor enzyme, poly(ADP-ribose) polymerase-1 (PARP-1) also becomes activated upon sensing DNA breakage. Activated PARP-1 cleaves NAD+ into nicotinamide and ADP-ribose and polymerizes the latter on nuclear acceptor proteins. Peroxynitrite-induced overactivation of PARP consumes NAD+ and consequently ATP culminating in cell dysfunction, apoptosis or necrosis [6,97]. PARP-1 activation has recently been implicated in the pathogenesis of diabetes and diabetic complications [48] including cardiovascular dysfunction [16,37,98–103], nephropathy [104], neuropathy [99,105] and retinopathy [106].

CONCLUSIONS AND IMPLICATIONS

Taken together, multiple lines of evidence support the view that nitrosative stress and peroxyntrite-induced damage play a crucial role in multiple interrelated aspects of the pathogenesis of diabetes and its complications. Neutralization of reactive nitrogen species or inhibition of downstream pathways including PARP activation may emerge as a novel approach for the experimental therapy of diabetes, as well as for the prevention or reversal of its complications.

ABBREVIATIONS

AGE
: Advanced glycation end product

AP-1
: Activator protein

DNA
: Deoxyribonucleic acid

eNOS
: Endothelial nitric oxide synthase

ET-1
: Endothelin-1

ICAM-1
: Intracellular adhesion molecule

iNOS
: Inducible nitric oxide synthase

NAD+
: Nicotinamide adenine dinucleotide

NT
: Nitrotyrosine (an index of nitrosative stress and Peroxynitrite formation)

$ONOO^-$
: Peroxynitrite

PARP/ PARS
: Poly(ADP-ribose) polymerase/synthase

PKC
: Protein kinase C

STZ
: Streptozotocin

T2DM
: Type 2 diabetes

T1DM
: Type 1 diabetes

VCAM-1
: Vascular cellular adhesion molecule

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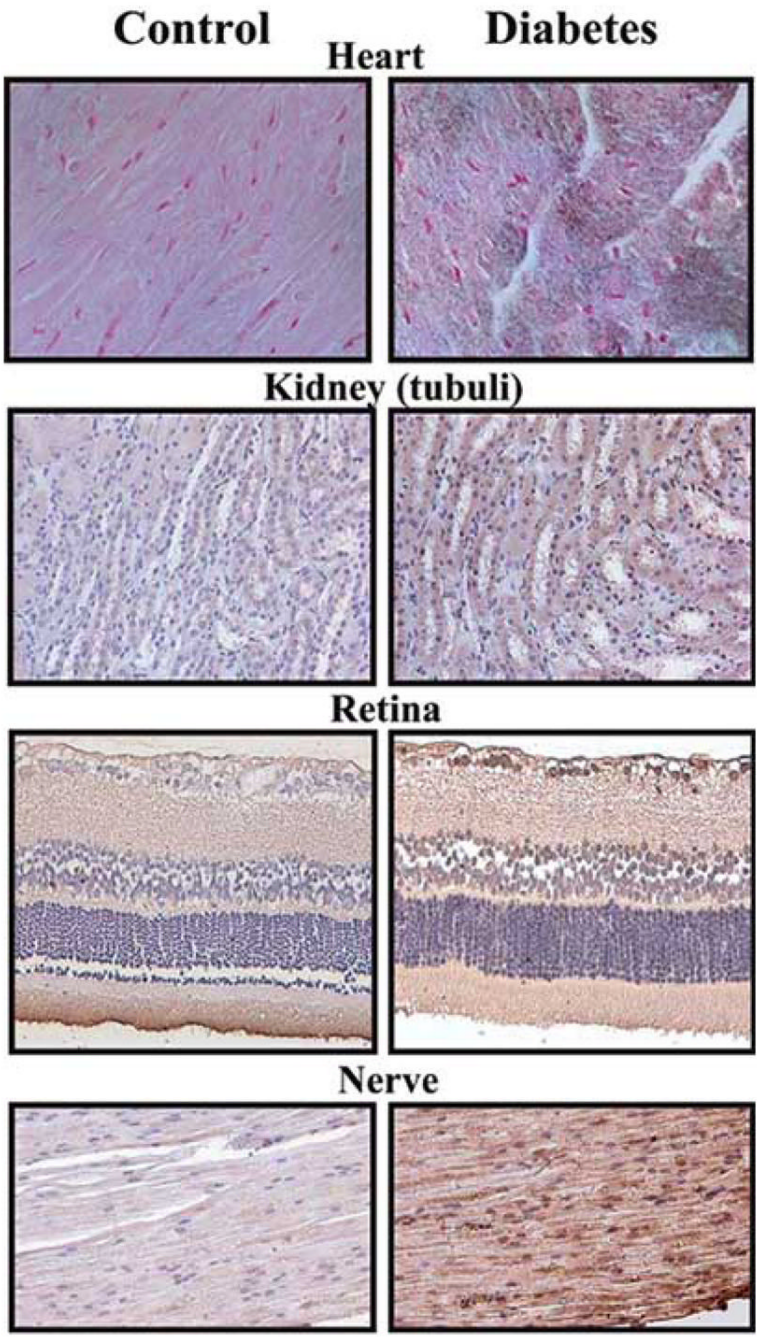

Fig. 1. Increased nitrotyrosine (NT) formation in diabetic tissues

Immunohistochemical staining for NT, an indicator of peroxynitrite formation, in control (left column), and 8 weeks old STZ-induced diabetic (right column) rat heart, kidney, retina and sciatic nerve tissue samples.

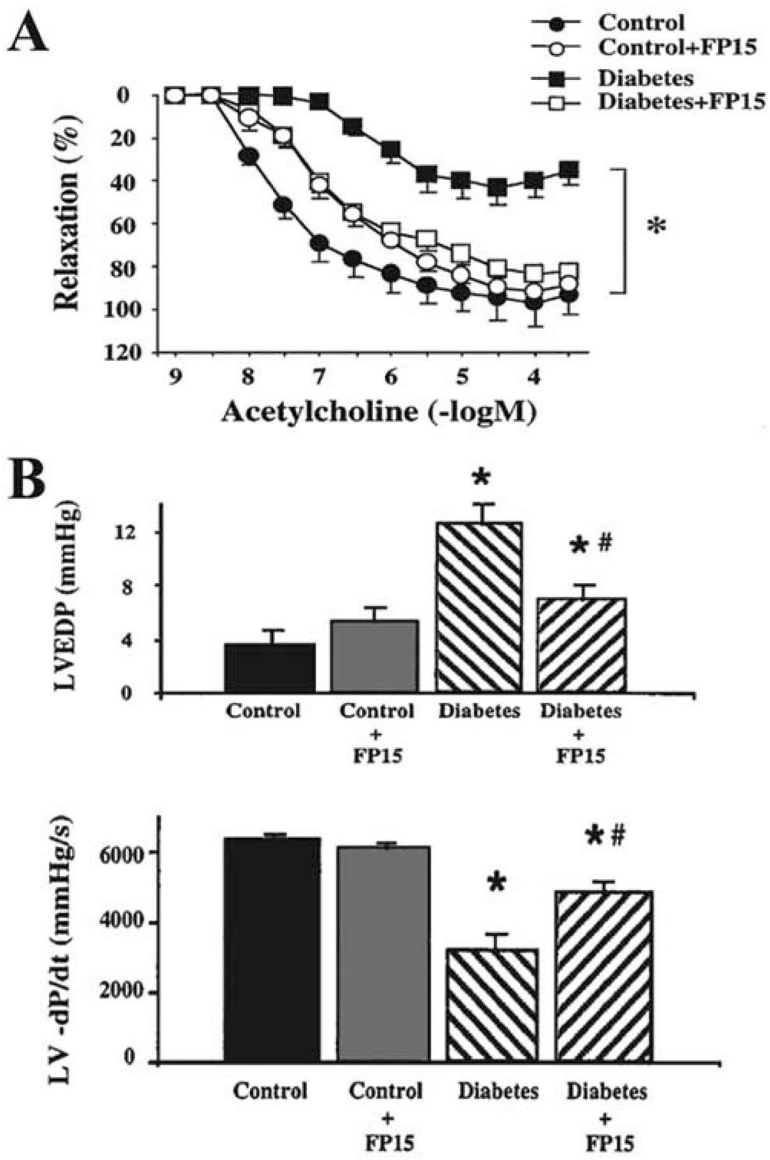

Fig. 2. Panel A. Reversal of diabetes-induced endothelial dysfunction by the porphyrinic peroxynitrite decomposition catalyst, FP15, in vascular rings from STZ-diabetic mice

Acetylcholine (Ach) induced endothelium-dependent relaxation is impaired in rings from diabetic mice, which is markedly improved by FP15 treatment. Each point of the curve represents the mean ± SEM of 5–7 pairs of experiments in vascular rings. *p< 0.05 in FP15 treated diabetic mice versus vehicle-treated diabetic mice.

Panel B. Reversal of streptozotocin-evoked diabetes-induced diastolic cardiac dysfunction by the porphyrinic peroxynitrite decomposition catalyst, FP15, in mice.

Effect of diabetes (9–10 weeks) and FP15 treatment in diabetic mice on left ventricular end diastolic pressure (LVEDP) and left ventricular -dP/dt (LV -dP/dt). Results are mean ± SEM of seven experiments in each group. *p< 0.05 diabetic animals versus control; #p< 0.05 in FP15-treated diabetic mice versus vehicle-treated diabetic mice. Reproduced with permission from 18.

Table 1

Selected Cytotoxic Processes Initiated by Peroxynitrite, with Potential Relevance to Diabetic Complications

Action	Mechanism(s)
Cytosolic enzyme inhibition	Oxidation, nitration
Membrane pump inhibition	Oxidation, nitration
Antioxidant enzyme inhibition	Oxidation, nitration
Signal transduction pathway disturbances	Oxidation, nitration
DNA injury	Oxidation, nitration, deamination, adduct formation
Surfactant protein damage	Nitration
Metalloproteinase activation	S-glutoxidation of prometalloproteinases
Antioxidant enzyme depletion	Glutathione, cysteine oxidation
Inhibition of BH_4-dependent enzymes	Direct BH_4 oxidation
Inhibition of NAD-dependent enzymes	NAD oxidation, NAD depletion *via* PARP
Lipid peroxidation	Peroxidation
Oxidative chain reactions	Lipid peroxidation, generation of reactive alpha-oxoaldehydes from glucose
Mitochondrial dysfunction	Inhibition of cytochromes, NADH-COQ1, etc.
Upregulation of adhesion receptors	NF- κB activation
GAPDH inhibition	Multiple, including PARP activation
Protein kinase C activation	Multiple, including GAPDH inhibition *via* PARP activation
Active DNA fragmentation	Caspase activation
Calcium dysregulation	Dysfunctional calcium pumps and cell energetics
Cell Necrosis	Mitochondrial injury, energetic collapse, oxidation, nitration, antioxidant depletion, calcium dysregulation
Apoptosis	Mitochondrial injury, DNA injury, caspase activation, signal transduction disturbances, calcium dysregulation

(Please note that to date many of the mechanisms listed have been demonstrated *in vitro* but not in experimental or clinical diabetes *in vivo*).

Table 2

Evidence Implicating Nitrosative Stress and Peroxynitrite Formation in Diabetes and Diabetic Complications

Organ/ Tissue, function Investigated	Species/ cells	Disease Model, trigger	Main Finding	Reference
Plasma	Human	T2DM	Cardiopulmonary bypass induced greater oxidative and nitrosative stress in diabetic patients.	7
Plasma	Human	T2DM	Increased plasma nitrotyrosine levels in diabetic patients	8
Plasma	Human	T2DM	Increased plasma nitrite/nitrate levels in diabetic patients.	9
Plasma	Human	T2DM	Postprandial hypertriglyceridemia and hyperglycemia induced endothelial dysfunction in diabetic patients and increased plasma nitrotyrosine levels, which was attenuated by simvastatin treatment.	10
Plasma	Human	T2DM	Increased plasma nitrotyrosine levels in diabetic patients, which correlate with postprandial hyperglycemia.	11
Plasma	Human	T1DM	Increased plasma nitrite, nitrate and nitrotyrosine , which correlate with the insulin requirements of the diabetic patients.	12
Plasma	Human	T1DM	Increased plasma nitrite/nitrate, nitrotyrosine and elevated blood pressure in diabetic patients.	13
LDL	Human	T1DM	Incubation of human aortic endothelial cells with LDL from T1DM patients increased Na^+/K^+-ATPase and Ca^{2+}- ATPase activities, NOS activity and peroxynitrite production.	14
Platelets	Human	T1DM, T2DM	Increased iNOS derived peroxynitrite formation in diabetic platelets	15
Aorta, vascular function	Mouse	Mouse STZ-induced diabetes	Increased eNOS expression, nitrotyrosine formation and PARP activation in endothelium and vascular smooth muscle.	16
Skin microvasculature	Human	T2DM and prediabetic	Increased nitrotyrosine formation and PARP activation in endothelial cells of diabetic and prediabetic patients.	17
Aorta, cardiac and vascular function, pancreatic islet beta-cells	Mouse	STZ-induced diabetes.	A peroxynitrite decomposition catalyst improved vascular and cardiac function and protected against diabetes.	18
Human aortic endothelial cells	Human	High glucose	Increased ONOO- formation, tyrosine nitration and inhibition of prostacycline synthase.	19
Human umbilical vein endothelial cells	Human	Stable or intermittent high glucose	Stable or intermittent high glucose stimulated nitrotyrosine formation through PKC-dependent activation of NAD(P)H oxidase.	20
Human aortic endothelial cells	Human	High glucose	Glucose-induced activation of PKC resulted in Peroxynitrite formation and nitration of prostacyclin synthase.	21
Bovine endothelial cells	Bovine	Hyperglycemia (HG)	HG induced increased lipid peroxidation, increased superoxide and peroxynitrite formation, and PKC activity.	22
Aorta, liver, kidney	Rat	STZ-induced diabetes	Increased free radical and NO concentrations in the liver, kidney and aorta; increased $ONOO^-$ formation in aorta.	23
Aorta, vascular function	Rat	Zucker diabetic rats	Age-dependent increase of nitrotyrosine formation in the vasculature and development of endothelial dysfunction, which is attenuated by a peroxynitrite scavenger, ebselen.	24

Organ/ Tissue, function Investigated	Species/ cells	Disease Model, trigger	Main Finding	Reference
Cardiac myocytes	Human	T2DM, hypertension	Increased apoptosis, necrosis, angiotensine II and nitrotyrosine formation in myocytes.	25
Cardiac myocytes	Mouse	STZ-induced diabetes	Increased apoptosis, H_2O_2, .OH, angiotensine II and nitrotyrosine formation in myocytes, which is decreased by IGF-1 overexpression.	26
Heart mitochondria	Rat	STZ-induced diabetes	Increased nitration and inactivation of succinyl-CoA:3- oxoacid CoA-transferase (SCOT).	27
Heart	Rat	High glucose	Perfusion of isolated hearts with high glucose increased superoxide generation, NO, nitrotyrosine formation and iNOS expression.	28
Heart	Mouse	Alloxan-induced diabtes	Tyrosine nitration of mitochondrial proteins.	29
Pancreatic islet beta-cells	Mouse	NOD mice	Increased nitrotyrosine formation in pancreatic islet beta-cells.	30
Placenta	Human	T1DM	Increased nitration in vascular endothelium and villous stroma.	31
Placental vasculature	Human	T1DM, preeclampsia	Increased nitrotyrosine formation, attenuated vasoconstrictor and vasodilatory responses in diabetes and preeclampsia.	32
Kidney	Human	Patients with diabetic nephropathy	Increased nitrotyrosine immunostaining in renal tubuli of diabetic patients.	33
Kidney	Rat	STZ-induced diabetes	Increased superoxide and nitrotyrosine formation in renal cortex.	34
Kidney	Mouse	STZ-induced diabetes	Increased renal nitrotyrosine and advanced glycation end product formation, which is attenuated by ramipril or aminoguanidine.	35
Kidney	Rat	STZ-induced diabetes	Increased renal expression of p47phox, hydrogen peroxide production and nitrotyrosine formation.	36
Retina	Rat	STZ-induced diabetes	Increased nitrosative stress, which is attenuated by aminoguanidine.	37
Retina	Rat	BBZ/Wor rat model of NIDDM	Increased iNOS and nitrotyrosine immunoreactivity in diabetic retinas.	38
Retina	Rat	STZ-induced diabetes	Increased retinal lipid peroxidation and nitrotyrosine formation, which was only slightly attenuated by reinstitution of good glycemic control.	39
Retina	Rat	STZ-induced diabetes	Increased tyrosine nitration and expression of vascular endothelial growth factor contribute to the breakdown of the blood-retina barrier in diabetes.	40
Retinal endothelial cells		High glucose	High glucose induced increased nitrotyrosine formation in retinal endothelial cells, which was blocked by superoxide or peroxynitrite scavengers, NOS or aldose reductase inhibitors.	41
Peripheral nerves, epineurial arterioles, endoneurial blood flow	Rat	STZ-induced diabetes	Antioxidants reduced the production of superoxide and peroxynitrite in epineurial arterioles and improved endoneural blood flow.	42
Epineurial arterioles, endoneurial blood flow	Rat	STZ-induced diabetes	Antioxidant (M40403) reduced the production of superoxide and peroxynitrite in epineurial arterioles and improved endoneural blood flow.	43
Peripheral motor nerve function	Human	T1DM	Decreased motor nerve function in diabetic patients correlates with increased nitrosative stress.	44

Organ/ Tissue, function Investigated	Species/ cells	Disease Model, trigger	Main Finding	Reference
Peripheral sensory neurons	Rat	STZ-induced diabetes	Rise in cytoplasmic labeling of nitrotyrosine, PARP activation.	45
Peripheral nerves, epineurial arterioles, endoneurial blood flow	Rat	STZ-induced diabetes	Antioxidants reduced the production of superoxide and peroxynitrite in epineurial arterioles and improved endoneural blood flow.	46
Bladder	Rat	STZ-induced diabetes	Increased proteasomal activation and nitrotyrosine formation during diabetic cystopathy.	47