

# OXIDATIVE STRESS CONTRIBUTIONS TO CHRONIC COMPLICATIONS IN DIABETES

OCTAVIAN SAVU and CONSTANTIN IONESCU-TÎRGOVIȘTE

”N. C. Paulescu” National Institute of Diabetes, Nutrition and Metabolic Diseases 1st Clinic  
5–7 Ion Movilă Street 79811, Bucharest 2 Romania  
E-mail addresses: savu.octavian@gmail.com; cit@paulescu.ro

*Received December 16, 2008*

Diabetes Mellitus is a serious global health problem. Both type 1 and type 2 diabetes markedly increase the risk of microvascular and macrovascular complications. Chronic complications of diabetes (retinopathy, nephropathy, neuropathy, and diabetes accelerated arteriosclerosis) represent a major medical and economical concern. Several pathogenic mechanisms were proposed to be responsible for the development of long-term complications of diabetes (*i.e.* increase polyol pathway flux, increased advanced glycation end products, activation of proteinkinase C, increased hexosamine pathway flux). A coherent pathogenic mechanism has just recently evolved by the discovery that each of the mechanisms mentioned reflects a single hyperglycemia-induced process: overproduction of reactive oxygen species by the mitochondria electron-transport chain. Oxidative stress is defined as tissue injury resulting from a disturbance in the equilibrium between the production free radicals and antioxidant defense mechanisms. In this review, we consider how glucose-induced oxidative stress may lead to microvascular and cardiovascular complications.

*Key words:* Diabetes; Chronic complications; Oxidative stress.

## INTRODUCTION

Diabetes Mellitus (DM) is a serious global health problem. A recent study by the World Health Organization estimated the worldwide prevalence of this disease is expected to grow from 171 million in 2000 to 366 million by 2030<sup>1</sup>. Both type 1 and type 2 diabetes markedly increase the risk of microvascular and macrovascular complications.

Microvascular complications, which center on dysfunction in the capillary bed of tissues, are wide-ranging, and include the retinopathy, nephropathy, and neuropathy that eventually affect nearly all patients with DM. Diabetic retinopathy (DR) is the major cause of adult blindness in many geographic areas<sup>2</sup>. Diabetic neuropathy (DN), which affects roughly half of all diabetic patients, is the most common cause of nontraumatic amputations<sup>3</sup> and diabetic nephropathy (DNf) is the major cause of end-stage renal disease<sup>4</sup>.

Macrovascular complications due to atherosclerosis remain the leading cause of death in diabetic patients. Myocardial infarction, stroke, and peripheral vascular disease are two to four times more prevalent in these patients. Moreover, atherosclerosis occurs earlier, and follows a more aggressive course<sup>5</sup>. Thus, the cardiovascular event rate in diabetic patients without documented coronary artery disease (CAD) is equivalent to that of nondiabetic patients with CAD<sup>5</sup>. Diabetic patients have higher mortality following myocardial infarction than nondiabetic subjects<sup>5</sup>. Women with diabetes lose their premenopausal cardioprotection, and are vulnerable to CAD at the same rate as men<sup>6</sup>.

Hyperglycemia, a characteristic feature of DM, predisposes to vascular complications, both microvascular as well as macrovascular, and an early indicator of such damage is endothelial dysfunction<sup>7</sup>. Several pathogenic mechanisms have

been proposed to be responsible for the development of long-term complications of diabetes: increase polyol pathway flux<sup>8</sup>, increased advanced glycation end products (AGEs)<sup>9</sup>, activation of protein kinase C (PKC)<sup>10, 11</sup>, increased hexosamine pathway flux<sup>12</sup>, increased oxidative stress<sup>13</sup>, carbonyl stress<sup>14</sup>, reductive stress (pseudohypoxia)<sup>15, 16</sup>, true hypoxia<sup>17</sup>, altered lipoprotein metabolism<sup>18, 19</sup>, and altered growth factor<sup>20</sup> or cytokine<sup>21</sup> activities.

A coherent pathogenic mechanism has just recently evolved by the discovery that at least three of the above mentioned mechanisms (i.e. increase polyol pathway flux, increased AGEs, activation of PKC) reflects a single hyperglycemia-induced process: overproduction of ROS by the mitochondria electron-transport chain<sup>22</sup>. To determine the site of hyperglycemia-induced intracellular ROS production, bovine aortic endothelial cells were incubated in 5mM glucose, 30 mM glucose alone and 30mM glucose plus either thenoyltrifluoroacetone (TTFA, a complex II inhibitor), carbonyl cyanide m-chlorophenylhydrazine (CCCP, an uncoupler of oxidative phosphorylation that abolishes the mitochondrial membrane proton gradient), uncoupling protein-1 (UCP1) or manganese superoxide dismutase (Mn-SOD) HVJ-liposomes (last two conditions inducing over expression for both UCP-1 or Mn-SOD). TTFA, CCCP or over expressed UCP-1 and Mn-SOD prevented the effect of hyperglycemia. These results show that hyperglycemia-induced intracellular ROS are produced by the proton electrochemical gradient generated by the mitochondrial electron transport chain, and that superoxide is the reactive oxygen radical produced by this mechanism. Moreover, it has been shown that hyperglycemia-induced overproduction of mitochondrial superoxide induces an important reversible decrease in glyceraldehyde phosphate dehydrogenase (GAPDH) activity. Superoxide induces this effect either direct<sup>23</sup> or indirect, *via* poly(ADP-ribose) polymerase (PARP) activation by oxidative lesions of mitochondrial DNA<sup>24</sup>. Despite the modulation, superoxide GAPDH inhibition induces four of the pathogenic mechanisms of hyperglycemic damage: hexosamine pathway, polyol pathway, advanced glycation end products production, and PKC pathway.

Under hyperglycemic condition, two major sources of excessively ROS (*i.e.* superoxide) production are described in mitochondrial

respiratory chain: complex I (NADH:ubiquinone oxidoreductase or NADH/FADH<sub>2</sub> dehydrogenase) and complex III (ubiquinol:cytochrome c oxidoreductase)<sup>25-27</sup>.

Based on large trials, it is well established that both the degree of glycemic control and the duration of diabetes predict the risk of diabetic complications, and that strict glycemic control dramatically lowered the incidence of retinopathy, nephropathy, and neuropathy. Nowadays, it is also widely accepted a variable degree of genetic predisposition for chronic complications occurrence in diabetes. Moreover, recently<sup>28</sup> the theory of “metabolic memory” was postulated. Basically, the concept defines an unknown pathophysiological mechanism responsible for a long-term, beneficial effect on the subsequent risk of microvascular and macrovascular complications following a period of intensive diabetes therapy.

All these data clearly demonstrates that microvascular and macrovascular diseases share a common pathophysiology, where oxidative stress plays a crucial role. Whereas tissue-specific factors may accentuate diabetic damage, we further discuss how oxidative stress may interfere with tissue-targeted hyperglycemic damage.

**Cardiovascular disease** is the major cause of morbidity and mortality in diabetes. About 80% of all diabetics die from cardiovascular events. 75% of such deaths are due to CAD and the remaining – due to cerebrovascular, peripheral or other macrovascular disease<sup>5, 29</sup>. The common link in these complications is accelerated atherosclerosis, a hallmark of endothelial dysfunction in diabetes<sup>30</sup>.

Oxidative stress in diabetic patients leads to many proatherogenic events such as LDL oxidation or abnormalities of NO synthesis<sup>30</sup>. The major contributor to endothelial oxidative stress is the increased production of superoxide. This seems to occur *via* two principal sources: NAD(P)H oxidases and uncoupled eNOS. Hyperglycemia, AGEs, FFA, and oxidised LDL (oxLDL) have been shown to increase endothelial NAD(P)H oxidase activity. The activation of NAD(P)H oxidases by hyperglycemia and FFA has been shown to be mediated by PKC<sup>31</sup>. Not only excess superoxide itself, but also its reaction with nitric oxide (NO) to produce peroxynitrite, can oxidize tetrahydrobiopterin (BH<sub>4</sub>)<sup>32</sup>, thus reducing its availability to eNOS. In the presence of reduced concentrations of BH<sub>4</sub>, eNOS becomes uncoupled and transfers electrons to molecular oxygen instead of L-arginine to produce superoxide rather than

NO. The presence of uncoupled eNOS in the diabetic vasculature is supported by either *in vitro* or *in vivo* studies<sup>33-35</sup>.

It is generally agreed that oxLDL is produced *in vivo* and that it contributes to diabetic atherosclerosis<sup>36-38</sup>. Extensive ROS production in diabetic vasculature makes LDL more easily to be oxidized. Moreover, excessive glycation in diabetics makes LDL more prone to oxidation<sup>39, 40</sup>. Oxidized LDL has itself been shown to produce oxidative stress in endothelial cells via activation of a NADPH oxidase<sup>41</sup>. The effect of oxLDL signaling may also be increased through the hyperglycemia induced up regulation of its receptor, both in endothelia and macrophages. As potential mediators oxidative stress, PKC and NF- $\kappa$ B are considered<sup>42, 43</sup>. In addition, the rate of LDL transvascular transport is higher in diabetics<sup>44</sup>. For all these reasons, oxLDL are considered highly aggressive for endothelial cells in diabetic condition.

Superoxide overproduction from endothelial cells in diabetes is a very important cause of NO deficiency. Uncoupled eNOS is essential for a defective NO synthesis. In addition, there are experimental data suggesting several inhibitory mechanisms for eNOS activity in diabetes mediated by oxidative stress. Activation of the hexosamine pathway is responsible for a specific modification of serine 1177 on eNOS which prevents its phosphorylation<sup>45</sup>. Oxidative stress induces serine phosphorylation of insulin receptor substrate-1 (IRS-1) and targets it for degradation. The decrease in IRS-1 leads to the impaired activation of the phosphatidylinositol 3-kinase/Akt pathway, contributing to eNOS inactivation<sup>46</sup>. Hyperglycemia also induces accumulation of asymmetric dimethylarginine (ADMA), an inhibitor of eNOS<sup>47</sup>.

Oxidative stress is also important for other several pro-atherosclerotic events occurring in diabetes, such as: increased inflammation, platelet activation, monocytes recruitment in atherosclerotic plaque, and vascular smooth muscle cells (VSMCs) proliferation and migration.

There is evidence suggesting that hyperglycemia causes oxidative stress in monocyte/macrophages, resulting in increased production of proatherogenic agents. For example, a single oral dose of glucose has been shown to increase ROS generation in monocytes of healthy volunteers<sup>48</sup>. Sixteen normal healthy adult volunteers were given either vodka (10 subjects), glucose solution

(10 subjects), or 300 mL water (7 subjects). Vodka and glucose drinks were equivalent to 300 calories. ROS generation at 1 hour, 2 hours, and 3 hours following ingestion was measured. ROS generation by both monocytes and neutrophils increased significantly ( $P < 0.05$  for monocytes and  $P < 0.01$  for neutrophils) following intake of glucose solution, but did not change significantly following alcohol or water. Furthermore, monocytes isolated from diabetic patients produce increased levels of superoxide through PKC dependent activation of NAD(P)H oxidase<sup>49</sup>. Hyperglycemia can increase monocyte adhesion by the increased expression of Monocyte Chemoattractant Protein 1 (MCP-1) *via* p38 mitogen-activated kinase and through the activation of h1-integrin by interleukin-8 and ROS from a mitochondrial source.<sup>50,51</sup> Monocyte invasion and VSMC migration may be facilitated by the ROS-mediated expression of MMP-9, a 92 kD metaloproteinase degrading collagen IV from extracellular matrix, which has been shown to be induced by glucose. Specific activity and expression of MMP-9 were significantly increased in vascular tissue and plasma of two distinct rodent models of DM, when compared with their controls<sup>52</sup>. When exposed to chronic hyperglycemia, monocytes secrete tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) *via* ROS-dependent activation of NF- $\kappa$ B.<sup>53</sup> NF- $\kappa$ B activity in diabetes is strongly conditioned by glycemic control. In this aspect, there are several *in vivo* studies where patients with type 1 DM were included, showing that monocytes of patients with either poorly controlled (HbA1c > 10%) or newly diagnosed diabetes have increased NF- $\kappa$ B activation<sup>54, 55</sup>.

As in endothelial cells, hyperglycemia has been shown to increase superoxide production in VSMC *via* NAD(P)H oxidase activation<sup>56-58</sup>. This increased superoxide could further react with NOS from endothelial cells, thus limiting its effect on VSMC relaxation. The production of NOS in VSMC themselves may be affected as well, because high glucose concentrations can inhibit iNOS activity in these cells through a PKC-dependent mechanism<sup>59</sup>. Both hyperglycemia and oxidative stress further impair endothelial vasodilation by shifting VSMC from a contractile to a proliferative phenotype. There are a number of studies showing that either on humans or animals. Many substances are cited as possible mediators for this phenomenon<sup>30</sup>: NAD(P)H oxidase, aldose-reductase (AR), NO deficiency in VSMC.

Interestingly, despite increased VMSC migration and proliferation observed in diabetic endothelium, atherosclerotic lesions contain very few VMSC in these patients. The explanation is provided by the observation that in aorta and coronary arteries from diabetic patients there is an increased rate of apoptosis and necrosis which are mediated by high level of hydrogen peroxide radicals and oxLDL<sup>60, 61</sup>. Increased VMSC death observed in diabetic patients has been proposed to play a potentially role in plaque instability and subsequent rupture<sup>62</sup>.

Nevertheless, it has to be referred several clinical studies showing improvement of endothelial functions in diabetic patients after antioxidants supplementation (*i.e.* vitamin E and N-acetyl cysteine, NAC)<sup>30</sup>. These data can be considered as indirect proofs for the essential role played by oxidative stress in endothelial dysfunction and accelerated atherosclerosis observed in diabetic patients.

**Diabetic cardiomyopathy** is a specific entity firstly described by Rubler and colab. in 1972<sup>63</sup>. In most of the cases, the disease is diagnosed as an impaired diastolic function in presence of hypertension or myocardial ischemia. The exact cause of the diabetic cardiomyopathy remains unclear, and the pathophysiology is multifactorial. Recent studies suggest a pathogenic role of free-radical mediated apoptosis<sup>64, 65</sup>. Hyperglycemia, hyperlipemia, and inflammation are important triggers for apoptosis. Based on current knowledge obtained from the studies of myocardium from intact diabetic animals and using cultured cardiomyocytes exposed to high levels of glucose and free fatty acid, several possible mechanisms for the induction of myocardial cell death by diabetes have been proposed<sup>64-66</sup>. Up regulation of the local renin-angiotensin system (RAS) and the synthesis of angiotensin II (Ang II) are one mechanism implicated in the induction of myocyte apoptosis in both type 1 diabetic animals and type 2 diabetic patients. Ang II binds to Ang II receptor 1 (AT1), activating a series of responses including the generation of reactive oxygen and nitrogen species (ROS and RNS) through activation of the NADH/NADPH oxidase. The evidence that myocardial cell death in diabetes could be prevented by application of AT1 blockers confirmed the involvement of this mechanism. ROS and RNS activate other proapoptotic mechanisms in cardiomyocytes, too<sup>64</sup>: mitochondrial cytochrome c - caspase-3 pathway, TNF- $\alpha$ , PKC, p38 MAP kinase, and PARP.

ROS/RNS involvement in all these above mentioned processes makes oxidative stress an important factor in cardiomyopathy pathophysiology.

**Diabetic nephropathy (DNf)** is characterized by persistent albuminuria, confirmed on at least two occasions 3–6 months apart, declining glomerular filtration rate (GFR), and hypertension. About 30% of all diabetics develop DNf at some point. DNf develops in ~35% of patients with type 1 DM and 15%–20% of patients with type 2 DM. Albuminuria correlates with risk of renal failure, as well as with cardiovascular events and mortality in type 2 DM patients<sup>65, 67</sup>.

Oxidative stress injury in DNf is potentially mediated by multiple factors<sup>65, 68, 69</sup>. Among these, three are considered to be very important: increased free radical production due to mitochondrial dysfunction, overproduction of AGEs, and abnormal NO synthesis and action. Increased growth factor activity, the activation of cytokines, and decreased glycosaminoglycan content in basement membranes, are also important in this scenario<sup>70</sup>. Additional evidence comes from studies showing increased urinary levels of 8-OHdG in type 2 diabetics as compared to controls, and this increase was directly proportional to the severity of glomerular and tubulointerstitial lesions<sup>71</sup>. Moreover, a 5-year prospective Japanese trial, documented a urinary 8 level of an 8-OHdG metabolite as a strong predictor of the development of DNf in type 2 diabetic patients<sup>72</sup>. In addition, it has been shown that either taurine, glutathione, or NAC decreased in a dose – dependent manner the apoptosis degree in human tubular cells exposed for 48 h to glucose 30 mM<sup>73</sup>. In the same study, taurine significantly reduces intracellular levels of hydrogen peroxide evaluated by fluorometry.

The generation of ROS, specifically superoxide, by hyperglycemia damaged or dysfunctional mitochondria, has been postulated as the primary initiating event in the development of diabetes complications<sup>22</sup>. This concept has only been validated in vitro on human mesangial cells long-term exposed to hyperglycemia<sup>74, 75</sup>. Despite of a strong argumentation, there is convincing in vivo evidence for dysfunctional mitochondria implications in DNf. A role for mitochondria in the development of diabetic kidney disease is further strengthened by the recent observation that up to 50% of children with mitochondrial diseases have renal impairment<sup>76</sup>. Some of these subjects have demonstrated renal disease as their primary pathology, and associate a newly described

mitochondriopathy involving a deficiency in coenzyme Q10<sup>77</sup>. Moreover, treatment with new generation antioxidant with selective uptake into mitochondria (i.e. idebenone or MitoQ) in these cases seems to be efficient. However, the efficacy of these relatively selective mitochondrial antioxidants in DNf remains to be determined<sup>78, 79</sup>.

In diabetes, uncoupling of NOS due to restricted substrate (L-arginine) availability or the absence of cofactors, is thought to generate superoxide in preference to NO. Indeed, one study in experimental DNf has suggested that uncoupling of NOS and NADPH oxidase provides two major sources of glomerular superoxide<sup>80</sup>. In that study, restoration of physiological levels of BH4 attenuated ROS production and improved renal function, suggesting that BH4 deficiency modulates the effect. Moreover, mouse studies of inducible nitric oxide synthase (iNOS) suggest that NO modulates glomerulosclerosis and tubulointerstitial fibrosis<sup>65, 81</sup>. Several studies demonstrated an up- as well as down regulated expression of NO-synthases (NOS) in experimental DNf. It is still not yet specified whether the regulation and activity of NOS is changed in human DNf. Hohenstein and colab. investigated the eNOS and iNOS expression in kidney biopsies from patients with type 2 DM and nephropathy<sup>82</sup>. eNOS is upregulated during natural course of DNf, whereas iNOS has differential expression. In the early phase of the disease, iNOS has a similar pattern as eNOS, and thereby contributes to the increased endothelial NO production during DNf. This initial expression pattern changed during later stages when iNOS expression mainly localizes to invading inflammatory cells rather than to glomerular cells. In conclusion, despite controversies, the pattern of NO production in kidney can be correlated with the severity of diabetic renal disease. In early phases, NO production is increased and is mediated by constitutively expressed isoforms of NOS, and – at least in type 2 DM – by iNOS, too. During advanced renal disease NO deficiency occurs and probably involves all NOS isoforms.

The advanced glycation pathway is considered to be a key process in mediating tissue damage in both diabetic microvascular and macrovascular disease<sup>9</sup>. There are several clinical evidence linking AGEs with renal microangiopathy in DM. Studies have shown the presence of significantly increased serum and skin levels of AGE in relationship to progression of microalbuminuria to

overt nephropathy and severity of renal complications in diabetic patients, respectively<sup>65, 83</sup>. In addition to the circulation, AGEs have been found in increased concentrations at various sites of injury in diabetes, such as renal glomeruli and tubules<sup>84, 85</sup>. Also, strict glycemic control has been shown to attenuate free radical production and AGE accumulation in human diabetic glomeruli<sup>86</sup>.

The interaction between AGEs and oxidative stress can be expressed in both ways<sup>75</sup>. On one hand, oxidative stress can generate AGEs by several mechanisms: intracellular auto-oxidation of glucose to glyoxal, decomposition of early glycation (Amadori) products to 3-deoxyglucosone, and fragmentation of metabolites of the pentose phosphate pathway such as glyceraldehyde-3 phosphate and dihydroxyacetone phosphate to the reactive carbonyl methylglyoxal<sup>87</sup>. On the other hand, any of the Maillard reaction compounds, as well as AGE proteins themselves, can act as a possible source of ROS. Mechanisms responsible for AGE-induced free radical production include creation of catalytic sites for free radical generation, stimulation of NADPH oxidase, and depletion of glutathione peroxidase, a potent cellular antioxidant<sup>88</sup>.

The excessive generation of ROS, specifically superoxide radical, by damaged mitochondria, is considered as an essential source of AGEs under hyperglycemia condition<sup>22</sup>. Complex I and III from mitochondrial respiratory chain are now accepted as main sources for superoxide production. Two recent studies<sup>75, 89</sup> conducted on diabetic rats validate this concept in kidney. Superoxide production was significantly increased in mitochondria extracts from renal cortex of diabetic rats when compared with their controls, due to glycosilation of complex I and III proteins.

Another important source for ROS in kidney is NAD(P)H oxidase complex. In addition to residing in phagocytic cells, NAD(P)H oxidase is present in nonphagocytic renal cell types such as mesangial and proximal tubular cells, vascular smooth muscle cells, endothelial cells, and fibroblasts. When oxidative stress is present, i.e. in diabetes, all these sources generate high amounts of free radicals, and NAD(P)H system became an important extra mitochondrial source of ROS<sup>90</sup>. This is very well documented in numerous studies performed *in vitro* and *in vivo* with rats<sup>75</sup>.

Interaction between AGEs and their specific renal receptors is a very important aspect in DNf pathogenesis. Specifically, AGEs-RAGE interaction

is of a particular interest in this aspect. Transgenic diabetic mice over expressing RAGE rapidly develop glomerulosclerosis<sup>91</sup>. AGEs neutralization (either by RAGE knock-out or long-term administration of a RAGE-neutralizing antibody) confers renoprotection<sup>92,93</sup>. In addition, in the glomeruli of patients with DNf, RAGE expression is upregulated and positively correlates with AGE accumulation<sup>94</sup>.

**Retinopathy** is one of the most severe ocular complications of diabetes and is a leading cause of acquired blindness in young adults<sup>95</sup>.

**Diabetic retinopathy (DR)** is the most frequent cause of new cases of blindness among adults aged 20–74 years. Its prevalence correlates with duration of DM. RD occurs very seldom in patients under 10 years of age, and it bursts after puberty. During the first 20 years of the disease, virtually all patients with type 1 DM and >60% of the patients with type 2 DM develop retinopathy<sup>96</sup>. Glycemia is an important factor for occurrence and progression of DR. Poor glycemic control increases the risk and severity and a tight glycemic variation has opposite effects. The strongest predictor for development and progression of retinopathy is the duration of diabetes itself<sup>28, 96, 97</sup>.

The retina is highly rich in polyunsaturated lipid membranes, and oxygen consumption and glycolysis rate are increased. These particularities make the retina extremely vulnerable to free radical-mediated lipid peroxidation. Thiobarbituric acid-reacting substances (TBARS) assay have been found to be increased in type 1 and type 2 diabetic patients<sup>65, 98-100</sup>.

Animal studies have demonstrated that oxidative stress contributes not only to the development of DR but also to the resistance of retinopathy to reverse after good glycemic control is reinstated the metabolic memory phenomenon<sup>30</sup>. Resistance of diabetic retinopathy to reverse is probably attributed to accumulation of damaged molecules and ROS that are not easily removed even after good glycemic control is reestablished<sup>101</sup>.

Mitochondria superoxide production is an essential contributor to DR pathophysiology. Several studies are cited proving that all major metabolic pathways involved in chronic complications of DM (*i.e.* polyol, AGEs, hexosamine, PKC) are over active in diabetic retina<sup>100, 101</sup>. The concept of a unique link between all these pathways has also been recently documented in retina tissue under hyperglycemic

condition<sup>102</sup>. To assess possible oxidative damage in diabetic retinas *in situ*, Ola and colab. measured the GAPDH activity in freshly dissected rat retinas from 3-mo diabetic rats and their age-matched controls. In diabetic retinas, a significant decrease (22.3%) in GAPDH activity was observed compared with control retinas. Moreover, Kowluru and colab. reports a decrease GAPDH activity in the same animal model with incipient DR<sup>100</sup>. The mechanism behind it is still unclear, but main candidates are GAPDH glycosilation or nitrosilation. The complex III activity is reduced in the retinal mitochondria of diabetic mice and rats suggesting that complex III is the origin of excessive ROS production in retina<sup>103</sup>.

The activation of PKC is also considered as a major pathway involved in the pathogenesis of DR. Activated PKC can bring about a variety of changes characteristic of DR that include increasing vessel permeability, alteration of retinal blood flow, stimulation of neovascularization, and endothelial proliferation and apoptosis<sup>101</sup>. Regulating the action of several growth factors such as VEGF, IGF-1, and transforming growth factor  $\beta$  (TGF- $\beta$ ), has a particular significance. VEGF plays a pivotal role in DR and is implicated as the mediator and initiator of non-proliferative and proliferative diabetic retinopathies, respectively<sup>104, 105</sup>. Retinal expression of VEGF is elevated by ROS, and VEGF can also interact with other metabolic pathways important to the development of retinopathy such as PKC, AGEs and the polyol pathway<sup>101</sup>. IGF-1 can have direct mitogenic effects and it can stimulate glucose transport into retinal microvascular endothelial cells via activation of PKC. It can also modulate the expression and activity of VEGF<sup>101</sup>. Similar to VEGF, the activation of IGF-1 also increases DAG levels and PKC activation. Although the exact role of IGF-1 in the pathogenesis of diabetic retinopathy remains to be elucidated, it is possible that IGF-1 can be modulated by oxidative stress via PKC pathway<sup>101</sup>.

AGEs implications in DR pathogenesis has been recently reviewed<sup>106</sup>. CML and other AGEs have been localized to retinal blood vessels in patients with type 2 DM and found to correlate with the degree of retinopathy. When nondiabetic animals are infused with preformed AGE albumin, the adducts accumulate around and within the pericytes, colocalize with AGE-Rs, induce basement membrane thickening, and contribute to the breakdown of the inner blood-retinal barrier.

Furthermore, retinal vascular endothelial cells exposed to AGEs show abnormal endothelial nitric oxide synthase expression, which may account for some of the vasoregulatory abnormalities seen in the retinal microcirculation in diabetes. *In vitro* studies have also demonstrated the up-regulation of VEGF in retinal cells after exposure to AGEs, potentially promoting retinal neovascularization and increasing permeability to proteins across the retinal barrier.

Redox imbalance in diabetic retina is another important source of ROS. Hyperglycemia induced polyol pathway activation depletes retinal NAD(P)H storage. Glutathione-synthase NAD(P)H dependent activity is depressed and the same glutathione synthesis. Moreover, superoxide dismutase (SOD) activity is decreased in the diabetic retina<sup>107</sup>. This leads to ischemic retinopathy and increased production of the superoxide<sup>108</sup>.

Oxidative stress contributes to both functional and structural changes in the retina microvasculature<sup>100</sup>. Structural changes range from basement membrane thickening and microvascular cell loss to capillary closure and acellular capillary formation. ROS mediates these changes by both direct and indirect mechanisms. Structural changes may both contribute to and result from functional changes such as altered blood flow, loss of intercellular junctions, and increased vessel permeability. ROS are shown to contribute to the blood–retinal barrier breakdown and alterations in retinal blood flow, some of the earliest functional changes observed in the pathogenesis of DR. Peroxynitrite synthesis resulted from direct reaction between superoxide and NO has a particular significance. Peroxynitrite induces apoptosis by direct oxidative alterations on internal mitochondrial membrane. Kowluru and Chan<sup>101</sup> observe increased peroxynitrite concentrations in the retina from diabetic rats with incipient DR which persists after 14 months.

Accelerated apoptosis of pericytes and endothelial cells occurs early in diabetes. Oxidative stress can induce apoptosis by multiple mechanisms<sup>100</sup> many of which have been observed in hyperglycemia and DR. Critical points in oxidative stress induced capillary cell apoptosis include the activation of NF- $\kappa$ B and caspases (*i.e.* 3 and 9). Activation of NF- $\kappa$ B is essential for hyperglycemia induced apoptosis in endothelial cells. In diabetes, it leads to initiation of a pro-apoptotic program in retina endothelial cells and

pericytes<sup>109</sup>. Pathways leading to caspase activation are initiated both in the cytosol and mitochondria with oxidative stress playing a role in both.

Further evidence implicating oxidative stress in the pathogenesis of DR comes from studies showing antioxidants (*i.e.* vitamin E) normalize preclinical DR<sup>65</sup>. Additionally, increased expression of mitochondrial SOD leads to decreased superoxide formation leading to prevention of apoptosis in the retina and its capillaries.

Oxidative stress also significantly contributes to the pathogenesis of diabetic cataract. The major contributors in the genesis of free radicals in diabetic lens are glycooxidation and glutathione depletion. Moreover, studies have documented the role of aldose reductase (AR) and sorbitol dehydrogenase (SDH), enzymes involved in polyol pathway, in the pathogenesis of slowly evolving diabetic cataract. This is thought to occur due to disequilibrium between free radicals and antioxidant defenses resulting from depletion of NADPH and NAD<sup>+</sup>, cofactors for both enzymes.

**The diabetic neuropathies (DN)** are heterogeneous, affecting different parts of the nervous system that present with diverse clinical manifestations. Most common among the neuropathies are chronic sensorimotor distal symmetric polyneuropathy (DPN) and the autonomic neuropathies (DAN). DPN is a diagnosis of exclusion<sup>110</sup>. Although exact prevalence depends on the diagnostic criteria used to identify neuropathy, most studies suggest that 50% of patients with a 20 years history of diabetes, of both type 1 and type 2, have neuropathy<sup>111</sup>. Around 10% of these cases of neuropathy are associated with abnormal sensations and pain. The prevalence of diabetic (DAN) varies between 1,6 and 90% in accordance with diagnostic modalities and diabetic population. Big trials (*i.e.* DCCT, Stockholm Diabetes Study, or Oslo Study – for type 1 DM, and Kumamoto Study for type 2 DM) show that the incidence of neuropathy increases with duration of diabetes and is accelerated by poor glycemic control. On the other hand, a good glycemic control improves DPN, especially in type 1 DM.

Hyperglycemia plays a major role in the development and progression of diabetic complications, including neuropathy. The key mechanism, implicated in the neural degeneration induced by hyperglycemia, is increased oxidative

stress associated with mitochondrial dysfunction, increased polyol pathway activity, increased PKC activity, and AGE accumulation. The role of oxidative stress in neuronal degeneration has been documented in multiple studies, especially in animal models<sup>65</sup>. This has been documented in peripheral nerve, dorsal root and sympathetic ganglia, and the vasculature of the peripheral nervous system<sup>112</sup>.

Growing evidence indicates that AR, the limiting enzyme in polyol pathway, has a key role in oxidative stress production in DN. The enzyme aldose reductase converts toxic aldehydes to inactive alcohols. At high glucose concentrations AR converts glucose to sorbitol, initiating the polyol pathway of glucose conversion to fructose. Two distinct, and potentially synergic, biochemical consequences of increased metabolic flux of glucose through the sorbitol pathway have been invoked to explain the deleterious effects of hyperglycemia on the diabetic nerve. On one hand, sorbitol accumulation induces compensatory depletion of taurine and myoinositol. There is a strong experimental support in this aspect. Studies with specific aldose reductase inhibitors (ARIs) in experimental DN have shown that ARIs ameliorate nerve-fiber damage and loss, and diabetic mice over-expressing human AR develop severe functional and structural abnormalities in peripheral nerves<sup>112</sup>. However, most of the early clinical trials with ARIs have been disappointing, with a lack of efficacy and unacceptable side effects. The promise shown with the newer ARIs is being currently explored. The potent ARI fidarestat has shown therapeutic benefit both in STZ rats and in humans. Fidarestat-treated diabetic patients showing significant improvement in symptoms and electrophysiological measures compared with the placebo group<sup>112</sup>. Studies in rodents using sorbitol dehydrogenase inhibitors (SDIs) have revealed conflicting results. On the other hand, exaggerate consumption of AR and SDH cofactors induce neuronal redox disequilibrium. NADPH to NADP oxidation leads to NADPH deficiency. NADPH is a common cofactor for AR, glutathione reductase and NOS. Glutathione reductase depletion enhances oxidative stress in peripheral nerves and lens. NOS depletion is responsible for decreased endoneurial nutritive blood flow<sup>113</sup>. Recent human genetic and biochemical data link polymorphisms of the AR gene to increased risk of diabetic complications<sup>114</sup>.

The activation of the PKC pathway exerts its negative action in DN through its effects in vascular blood flow and microvascular disease rather than directly in neuronal cells<sup>111,112</sup>. Increases in PKC activity within the vasa nervorum lead to increased contractile responsiveness and diminished vasodilation, suggesting that PKC inhibition should enhance perfusion and improve nerve dysfunction. It seems that in neurons the PKC functional pattern is different. On one side there are reports showing that endoneurial PKC activity was significantly reduced. On the other – ones show that epineurial PKC activity was increased and that changes in PKC activity are different between the membrane fraction and the cytosol<sup>115</sup>. In animal studies, treatment with a PKC inhibitor prevented the activation of NF- $\kappa$ B and subsequent ROS formation<sup>116</sup> and reduced the nerve conduction defects and increased endoneurial perfusion<sup>117</sup>. Together, these studies suggest that the positive effect of PKC inhibition on nerve conduction may be due to improved nerve blood flow rather than to an improvement of the nerve Na<sup>+</sup>/K<sup>+</sup>-ATPase defects.

It has been shown that the glycation process is enhanced in the peripheral nerve in both diabetic humans and animals<sup>118,119</sup>. The main source is fructose. Fructose can be metabolized to fructose-3-phosphate and triose phosphate by phosphokinase and fructokinase, respectively. These metabolites become potential sources of 3-deoxoglucosone (3DG) and methylglyoxal (MGO) both of which are main AGEs in neurons. As in case of other chronic complications, binding interactions between AGEs and their specific receptors have been suggested as a source of oxidative stress, by depleting intracellular GSH and vitamin C, resulting in the induction of nuclear factor kappa B (NF- $\kappa$ B) in endothelial cells<sup>120</sup>. In diabetic rat models, it has been shown that AG inhibits the formation of AGEs in aortic collagen and that has beneficial effects on the development of neuropathy<sup>121</sup>.

Endothelial poly(ADP-ribose) polymerase (PARP) activation is considered as essential for the development of endothelial dysfunction in DM<sup>122</sup>. Oxidant-induced DNA single-strand breaks under hyperglycemia condition activates PARP and depletes NAD<sup>+</sup> and ATP storages. Glycolysis and mitochondrial respiration are depressed and free radical production is increased. Reactive oxygen and nitrogen species activates PARP in diabetic



endothelial cells. This is sustained by the fact that hyperglycemia mediated PARP activation is depressed by SOD, NOS inhibitors or NO chelators. Importantly, pharmacological PARP inhibition can also restore sensory and motor neuronal conduction in already established diabetic neuropathy, at least in murine models of the disease<sup>123, 124</sup>.

Vincent and colab. demonstrated significantly elevated levels of oxidative stress in the dorsal root ganglia, within 2 h of hyperglycemia, leading to apoptosis. This may explain in part the underlying mechanism responsible for neuropathy in diabetics with good overall control as well as patients with impaired glucose tolerance, who also develop neuropathy<sup>125, 126</sup>.

Nevertheless, oxidative stress implications in pathogenesis of ND are also sustained by indirect evidence originated from studies using antioxidants<sup>112</sup>. From this perspective, most of the data are provided by studies using  $\alpha$ -lipoic acid (LA) also known as thioctic acid. Human studies in diabetic patients generally report significant improvements in antioxidants profile and decreases of oxidative stress, even in subjects with poor glycemic control<sup>127</sup>. Some other studies showed that treatment with LA tends to improve the microcirculation, in addition to decreasing cellular oxidative stress<sup>128</sup>. Larger multicenter, randomized, double-blind placebo trials have demonstrated limited effects on neuropathic symptoms and electrophysiological testing, proposing that a longer-term assessment of neuropathic deficits is merited (*i.e.* longer than 7 months)<sup>129, 130</sup>. Slight improvements in cardiac autonomic neuropathy were also demonstrated<sup>131</sup>. A recent phase III clinical trial, the SYDNEY trial, demonstrated that *i.v.* administration of LA rapidly and significantly improves several neuropathic symptoms and nerve function in patients with stage 2 DPN<sup>132</sup>.

## REFERENCES

1. Wild S., Roglic G., Green A., Sicree R., King H., *Global prevalence of diabetes: estimates for the year 2000 and projections for 2030*, Diabetes Care, **2004**, 27, 1047–1053.
2. Klein R., Klein B.E., Moss S.E., *Epidemiology of proliferative diabetic retinopathy*, Diabetes Care, **1992**, 15, 1875–91.
3. Feldman E.L., *Oxidative stress and diabetic neuropathy: a new understanding of an old problem*, J Clin Invest, **2003**, 111, 431–433.
4. Bo S., Ciccone G., Rosato R., *et al.*, *Renal damage in patients with type 2 diabetes: a strong predictor of mortality*, Diabet Med, **2005**, 22, 258–65.
5. Haffner S.M., *Coronary heart disease in patients with diabetes*, N Engl J Med, **2000**, 342, 1040–1042.
6. Hu F.B., Stampfer M.J., Solomon C.G., Liu S., Willett W.C., Speizer F.E., Nathan D.M., Manson J.E., *The impact of diabetes mellitus on mortality from all causes and coronary heart disease in women: 20 years of follow-up*, Arch Intern Med, **2001**, 161, 1717–1723.
7. Giugliano D., Ceriello A., Paolisso G., *Oxidative stress and diabetic vascular complications*, Diabetes Care, **1996**, 19, 257–267.
8. Lee A.Y., Chung S.K., Chung S.S., *Demonstration that polyol accumulation is responsible for diabetic cataract by the use of transgenic mice expressing the aldose reductase gene in the lens*, Proc.Natl.Acad.Sci.U.S.A., **1995**, 92 (7), 2780–2784.
9. Brownlee M., *Advanced protein glycosylation in diabetes and aging*, Annu Rev Med, **1995**, 46, 223–234.
10. Koya D., King G.L., *Protein kinase C activation and the development of diabetic complications*, Diabetes, **1998**, 47 (6), 859–866.
11. Ishii H., Daisuke K., King G.L., *Protein kinase C activation and its role in the development of vascular complications in diabetes mellitus*, J Mol Med, **1998**, 76, 21–31.
12. Sayeski P.P., Kudlow J.E., *Glucose metabolism to glucosamine is necessary for glucose stimulation of transforming growth factor-alpha gene transcription*, J Biol.Chem, **1996**, 271 (25), 15237–15243.
13. Baynes J.W., *Role of oxidative stress in development of complications in diabetes*, Diabetes, **1991**, 40 (4), 405–412.
14. Kennedy A.L., Lyons T.J., *Glycation, oxidation, and lipoxidation in the development of diabetic complications*, Metabolism, **1997**, 46 (12 Suppl 1), 14–21.
15. Williamson J.R., Chang K., Frangos M., Hasan K.S., Ido Y., Kawamura T., Nyengaard J.R., Enden M. van den, Kilo C., Tilton R.G., *Hyperglycemic pseudohypoxia and diabetic complications*, Diabetes, **1993**, 42 (6), 801–813.
16. Ido Y., Kilo C., Williamson J.R., *Cytosolic NADH/NAD<sup>+</sup>, free radicals, and vascular dysfunction in early diabetes mellitus*, Diabetologia, **1997**, 40, S115–S117.
17. Cameron N.E., Cotter M.A., *Metabolic and vascular factors in the pathogenesis of diabetic neuropathy*, Diabetes, **1997**, 46, S31–S37.
18. Witztum J.L., *Role of modified lipoproteins in diabetic macroangiopathy*, Diabetes, **1997**, 46 Suppl 2, S112–S114.
19. Lopes-Virella M.F., Binzafar N., Rackley S., Takei A., La Via M., Virella G., *The uptake of LDL-IC by human macrophages: predominant involvement of the Fc gamma RI receptor*, Atherosclerosis, **1997**, 135 (2), 161–170.
20. Pfeiffer A., Schatz H., *Diabetic microvascular complications and growth factors*, Exp.Clin.Endocrinol. Diabetes, **1995**, 103 (1), 7–14.
21. Sharma K., Ziyadeh F.N., *Biochemical events and cytokine interactions linking glucose metabolism to the development of diabetic nephropathy*, Semin.Nephrol, **1997**, 17 (2), 80–92.
22. Nishikawa T., Edelstein D., Du X.L., Yamagishi S., Matsumura T., Kaneda Y., Yorek M.A., Beebe D., Oates P.J., Hammes H.P., Giardino I., Brownlee M., *Normalizing mitochondrial superoxide production blocks three pathways of hyperglycemic damage*, Nature, **2000**, 404 (6779), 787–790.
23. Du X.L., *et al.*, *Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation*, Proc. Natl Acad. Sci. USA, **2000**, 97, 12222–12226.

24. Soriano F.G., *et al.*, *Diabetic endothelial dysfunction: the role of poly(ADP-ribose) polymerase activation*, *Nature Med.*, **2001**, 7, 108–113.
25. Turrens J.F., Boveris A., *Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria*, *Biochem J.*, **1980**, 191(2), 421–7.
26. Beyer R.E., *An analysis of the role of coenzyme Q in free radical generation and as an antioxidant*, *Biochem Cell Biol.*, **1992**, 70(6), 390–403.
27. Turko I.V., Li L., Aulak K.S., Stuehr D.J., Chang J.Y., Murad F., *Protein tyrosine nitration in the mitochondria from diabetic mouse heart. Implications to dysfunctional mitochondria in diabetes*, *J Biol Chem.*, **2003**, 278(36), 33972–7.
28. DCCT/EDIC Research Group. *Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group*, *N Engl J Med*, 2000, 342, 381–389.
29. Rossi R., Nuzzo A., Grimaldi T., *et al.*, *Diabetes and cardiovascular disease: a close and dangerous connection*, *Heart Int.*, **2005**, 1, 18–23.
30. Jay D., Hitomi H., Griendling K.K., *Oxidative stress and diabetic cardiovascular complication*, *Free Rad Biol Med.*, **2006**, 40, 183–192.
31. Inoguchi T., Li P., Umeda F., Yu H.Y., Kakimoto M., Imamura M., Aoki T., Etoh T., Hashimoto T., Naruse M., Sano H., Utsumi H., Nawata H., *High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells*, *Diabetes*, **2000**, 49, 1939–1945.
32. Kossenjans W., Eis A., Sahay R., Brockman D., Myatt L., *Role of peroxynitrite in altered fetal-placental vascular reactivity in diabetes or preeclampsia*, *Am. J. Physiol. Heart Circ. Physiol.*, **2000**, 278, H1311–H1319.
33. Guzik T.J., Mussa S., Gastaldi D., Sadowski J., Ratnatunga C., Pillai R., Channon K.M., *Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase*, *Circulation*, **2002**, 105, 1656–1662.
34. Alp N.J., Mussa S., Khoo J., Cai S., Guzik T., Jefferson A., Goh N., Rockett K.A., Channon K.M., *Tetrahydrobiopterin-independent preservation of nitric oxide-mediated endothelial function in diabetes by targeted transgenic GTP-cyclohydrolase I overexpression*, *J. Clin. Invest.*, **2003**, 112:725–735.
35. Heitzer T., Krohn K., Albers S., Meinertz T., *Tetrahydrobiopterin improves endothelium-dependent vasodilation by increasing nitric oxide activity in patients with type II diabetes mellitus*, *Diabetologia*, **2000**, 43, 1435–1438.
36. Liguori A., Abete P., Hayden J.M., Cacciatore F., Rengo F., Ambrosio G., Bonaduce D., Condorelli M., Reaven P.D., Napoli C., *Effect of glycemic control and age on low-density lipoprotein susceptibility to oxidation in diabetes mellitus type I*, *Eur. Heart J.*, **2001**, 22, 2075–2084.
37. MacDonald-Wicks L., Gibson L.Z., Godfrey D.M., Green J.M., Horan B.P., Monger K.L., Wischer R.M., Garg M.L., *Oxidised LDL in newly diagnosed type 2 diabetes mellitus and impaired glucose tolerance*, *Asia Pac. J. Clin. Nutr.*, **2004**, 13(Suppl.), S65.
38. Ceriello A., Bortolotti N., Motz E., Pieri C., Marra M., Tonutti L., Lizzio S., Feletto F., Catone B., Taboga C., *Meal-induced oxidative stress and low-density lipoprotein oxidation in diabetes: the possible role of hyperglycemia*, *Metab. Clin. Exp.*, **1999**, 48, 1503–1508.
39. Basta G., Schmidt A.M., De Caterina R., *Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes*, *Cardiovasc. Res.*, **2004**, 63, 582–592.
40. Knott H.M., Brown B.E., Davies M.J., Dean R.T., *Glycation and glycooxidation of low-density lipoproteins by glucose and lowmolecular mass aldehydes: formation of modified and oxidized particles*, *Eur. J. Biochem.*, **2003**, 270, 3572–3582.
41. O'Donnell R.W., Johnson D.K., Ziegler L.M., DiMattina A.J., Stone R.I., Holland J.A., *Endothelial NADPH oxidase: mechanism of activation by low-density lipoprotein*, *J. Endothelial. Cell Res.*, **2003**, 10, 291–297.
42. Li L., Sawamura T., Renier G., *Glucose enhances endothelial LOX-1 expression: role for LOX-1 in glucose-induced human monocyte adhesion to endothelium*, *Diabetes*, **2003**, 52, 1843–1850.
43. Li L., Sawamura T., Renier G., *Glucose enhances human macrophage LOX-1 expression: role for LOX-1 in glucose-induced macrophage foam cell formation*, *Circ. Res.*, **2004**, 94, 892–901.
44. Kornerup K., Nordestgaard B.G., Feldt-Rasmussen B., Borch-Johnsen K., Jensen K.S., Jensen J.S., *Increased transvascular low density lipoprotein transport in insulin dependent diabetes: a mechanistic model for development of atherosclerosis*, *Atherosclerosis*, **2003**, 170:163–168.
45. Du X.L., Edelstein D., Dimmeler S., Ju Q., Sui C., Brownlee M., *Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site*, *J. Clin. Invest.*, **2001**, 108, 1341–1348.
46. Potashnik R., Bloch-Damti A., Bashan N., Rudich A., *IRS1 degradation and increased serine phosphorylation cannot predict the degree of metabolic insulin resistance induced by oxidative stress*, *Diabetologia*, **2003**, 46, 639–648.
47. Lin K.Y., Ito A., Asagami T., Tsao P. S., Adimoolam S., Kimoto M., Tsuji H., Reaven G.M., Cooke J.P., *Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase*, *Circulation*, **2002**, 106, 987–992.
48. Dhindsa S., Tripathy D., Mohanty P., Ghanim H., Syed T., Aljada A., Dandona P., *Differential effects of glucose and alcohol on reactive oxygen species generation and intranuclear nuclear factor-kappaB in mononuclear cells*, *Metab. Clin. Exp.*, **2004**, 53, 330–334.
49. Venugopal S.K., Devaraj S., Yang T., Jialal I., *Alpha-tocopherol decreases superoxide anion release in human monocytes under hyperglycemic conditions via inhibition of protein kinase C-alpha*, *Diabetes*, **2002**, 51, 3049–3054.
50. Takaishi H., Taniguchi T., Takahashi A., Ishikawa Y., Yokoyama M., *High glucose accelerates MCP-1 production via p38 MAPK in vascular endothelial cells*, *Biochem. Biophys. Res. Commun.*, 305, **2003**, 122–128.
51. Srinivasan S., Yeh M., Danziger E.C., Hatley M.E., Riggan A.E., Leitinger N., Berliner J.A., Hedrick C.C., *Glucose regulates monocyte adhesion through endothelial production of interleukin-8*, *Circ. Res.*, **2003**, 92, 371–377.

52. Uemura S., Matsushita H., Li W., Glassford A.J., Asagami T., Lee K.H., Harrison D.G., Tsao P.S., *Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress*, *Circ. Res.*, **2001**, 88, 1291–1298.
53. Guha M., Bai W., Nadler J.L., Natarajan R., *Molecular mechanisms of tumor necrosis factor alpha gene expression in monocytic cells via hyperglycemia-induced oxidant stress-dependent and -independent pathways*, *J. Biol. Chem.*, **2000**, 275, 17728–17739.
54. Hofmann M.A., Schiekofer S., Kanitz M., Klevesath M.S., Joswig M., Lee V., Morcos M., Tritschler H., Ziegler R., Wahl P., Bierhaus A., Nawroth P.P., *Insufficient glycemic control increases nuclear factor-kappa B binding activity in peripheral blood mononuclear cells isolated from patients with type 1 diabetes*, *Diabetes Care*, **1998**, 21:1310–1316.
55. Schiekofer S., Galasso G., Andrassy M., Aprahamian T., Schneider J., Rocnik E., *Glucose control with insulin results in reduction of NF-kappaB-binding activity in mononuclear blood cells of patients with recently manifested type 1 diabetes*, *Diabetes Obes Metab.*, **2006**, 8(5), 473–482.
56. Inoguchi T., Li P., Umeda F., Yu H.Y., Kakimoto M., Imamura M., Aoki T., Etoh T., Hashimoto T., Naruse M., Sano H., Utsumi H., Nawata H., *High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells*, *Diabetes*, **2000**, 49, 1939–1945.
57. Inoguchi T., Sonta T., Tsubouchi H., Etoh T., Kakimoto M., Sonoda N., Sato N., Sekiguchi N., Kobayashi K., Sumimoto H., Utsumi H., Nawata H., *Protein kinase C-dependent increase in reactive oxygen species (ROS) production in vascular tissues of diabetes: role of vascular NAD(P)H oxidase*, *J. Am. Soc. Nephrol.*, **2003**, 14(8 Suppl. 3), S227 – S232.
58. Lee H.S., Son S.M., Kim Y.K., Hong K.W., Kim C.D., *NAD(P)H oxidase participates in the signaling events in high glucose-induced proliferation of vascular smooth muscle cells*, *Life Sci.*, 72, **2003**, 2719 – 2730.
59. Muniyappa R., Srinivas P.R., Ram J.L., Walsh M.F., Sowers J.R., *Calcium and protein kinase C mediate high-glucose-induced inhibition of inducible nitric oxide synthase in vascular smooth muscle cells*, *Hypertension*, 31(1 Pt. 2), **1998**, 289–295.
60. Fukumoto H., Naito Z., Asano G., Aramaki T., *Immunohistochemical and morphometric evaluations of coronary atherosclerotic plaques associated with myocardial infarction and diabetes mellitus*, *J. Atheroscler. Thromb.*, **1998**, 5, 29–35.
61. Peiro C., Lafuente N., Matesanz N., Cercas E., Llergo J.L., Vallejo S., Rodriguez-Manas L., Sanchez-Ferrer C.F., *High glucose induces cell death of cultured human aortic smooth muscle cells through the formation of hydrogen peroxide*, *Br. J. Pharmacol.*, **2001**, 133, 967–974.
62. Hsieh C.C., Yen M.H., Yen C.H., Lau Y.T., *Oxidized low density lipoprotein induces apoptosis via generation of reactive oxygen species in vascular smooth muscle cells*, *Cardiovasc. Res.*, **2001**, 49:135–145.
63. Rubler S., Dlugash J., Yuceoglu Y.Z., Kumral T., Branwood A.W., Grishman A., *New type of cardiomyopathy associated with diabetic glomerulosclerosis*, *Am. J. Cardiol.*, 30, **1972**, 595–602.
64. Cai L., James Kang Y., *Cell Death and Diabetic Cardiomyopathy*, *Cardiovascular Toxicology*, **2003**, 03, 219–228.
65. Shah S., Iqbal M., Karam J., Salifu M., McFarlane S.I., *Oxidative Stress, Glucose Metabolism, and the Prevention of Type 2 Diabetes: Pathophysiological Insights, Antioxidants & Redox Signalling*, **2007**, 9(7), 911–929.
66. Cai L., Li W., Wang G., Guo L., Jiang Y., Kang Y.J., *Hyperglycemia-induced apoptosis in mouse myocardium: mitochondrial cytochrome C-mediated caspase-3 activation pathway*, *Diabetes*, **2002**, 51, 1938–1948.
67. Coccheri S., *Approaches to prevention of cardiovascular complications and events in diabetes mellitus*, *Drugs*, **2007**, 67(7), 997–1026.
68. Beisswenger P.J., Drummond K.S., Nelson R.G., Howell S.K., Szwegold B.S., Mauer M., *Susceptibility to diabetic nephropathy is related to dicarbonyl and oxidative stress*, *Diabetes*, **2005**, 54, 3274–3281.
69. Simmons R.A., *Developmental origins of diabetes: The role of oxidative stress*, *Free Radical Biol Med*, 40, **2006**, 917–922.
70. Caramori M.L.A., Mauer M., *Diabetes and nephropathy*, *Current Opinion Nephrol Hypertension*, **2003**, 12, 273–282.
71. Kanauchi M., Nishioka H., Hashimoto T., *Oxidative DNA damage and tubulointerstitial injury in diabetic nephropathy*, *Nephron*, **2002**, 91, 327–329.
72. Hinokio Y., Suzuki S., Hirai M., Suzuki C., Suzuki M., Toyota T., *Urinary excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine as a predictor of the development of diabetic nephropathy*, *Diabetologia*, **2002**, 45, 877–882.
73. Verzola D., Bertolotto M.B., Villaggio B., Ottonello L., Dallegrì F., Frumento G., Berruti V., Gandolfo M.T., Garibotto G., Deferran G., *Taurine prevents apoptosis induced by high ambient glucose in human tubule renal cells*, *J Investig Med*, **2002**, 50, 443–451.
74. Kiritoshi S., Nishikawa T., Sonoda K., Kukidome D., Senokuchi T., Matsuo T., Matsumura T., Tokunaga H., Brownlee M., Araki E., *Reactive oxygen species from mitochondria induce cyclooxygenase-2 gene expression in human mesangial cells: potential role in diabetic nephropathy*, *Diabetes*, **2003**, 52, 2570–2577.
75. Forbes J.M., Coughlan M.T., Cooper M.E., *Oxidative Stress as a Major Culprit in Kidney Disease in Diabetes*, *Diabetes*, **2008**, 57, 1446–1454.
76. Martin-Hernandez E., Garcia-Silva M.T., Vara J., Campos Y., Cabello A., Muley R., Del Hoyo P., Martin M.A., Arenas J., *Renal pathology in children with mitochondrial diseases*, *Pediatr Nephrol*, **2005**, 20, 1299–1305.
77. Diomedì-Camassei F., Di Giandomenico S., Santorelli F.M., Caridi G., Piemonte F., Montini G., Ghiggeri G.M., Murer L., Barisoni L., Pastore A., Muda A.O., Valente M.L., Bertini E., Emma F., *COQ2 nephropathy: a newly described inherited mitochondriopathy with primary renal involvement*, *J Am Soc Nephrol*, **2007**, 18, 2773–2780.
78. Hausse A.O., Aggoun Y., Bonnet D., Sidi D., Munnich A., Rotig A., Rustin P., *Idebenone and reduced cardiac hypertrophy in Friedreich's ataxia*, *Heart*, **2002**, 87, 346–349.
79. Green K., Brand M.D., Murphy M.P., *Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes*, *Diabetes*, **2004**, 53 Suppl 1, S110–8.

80. Satoh M., Fujimoto S., Haruna Y., Arakawa S., Horike H., Komai N., Sasaki T., Tsujioka K., Makino H., Kashiwara N., *NAD(P)H oxidase and uncoupled nitric oxide synthase are major sources of glomerular superoxide in rats with experimental diabetic nephropathy*, *Am J Physiol Renal Physiol*, **2005**, 288, F1144–F1152.
81. Trachtman H., Futterweit S., Pine E., Mann J., Valderama E., *Chronic diabetic nephropathy: role of inducible nitric oxide synthase*, *Pediatr Nephrol*, **2002**, 17, 20–29.
82. Hohenstein B., Hugo C.P.M., Hausknecht B., Boehmer K.P., Riess R.H., Schmieder R.E., *Analysis of NO-synthase expression and clinical risk factors in human diabetic nephropathy*, *Nephrol Dial Transplant*, **2008**, 23, 1346–1354.
83. Miura J., et al., *Serum levels of non-carboxymethyllysine advanced glycation endproducts are correlated to severity of microvascular complications in patients with Type 1 diabetes*, *J. Diabetes Complications*, **2003**, 17, 16–21.
84. Forbes J.M., et al., *Reduction of the accumulation of advanced glycation end products by ACE inhibition in experimental diabetic nephropathy*, *Diabetes*, **2002**, 51, 3274–3282.
85. Horie K., et al., *Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. Implication for glycoxidative stress in the pathogenesis of diabetic nephropathy*, *J. Clin. Invest.*, **1997**, 100, 2995–3004.
86. Suzuki D., Miyata T., Saotome N., Horie K., Inagi R., Yasuda Y., Uchida K., Izuhara Y., Yagama M., Sakai H., Kurokawa K., *Immunohistochemical evidence for an increased oxidative stress and carbonyl modification of proteins in diabetic glomerular lesions*, *J Am Soc Nephrol*, **1999**, 10, 822–832.
87. Thornalley P.J., Langborg A., Minhas H.S., *Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose*, *Biochem J*, **1999**, 344, 109–116.
88. Yagihashi S., *Pathogenetic mechanisms of diabetic neuropathy: lessons from animal models*, *J. Peripher. Nerv. Syst.*, **1997**, 2, 113–132.
89. Rosca M.G., et al., *Glycation of mitochondrial proteins from diabetic rat kidney is associated with excess superoxide formation*, *Am J Physiol Renal Physiol*, **2005**, 289, 420–430.
90. Griendling K.K., Minieri C.A., Ollerenshaw J.D., Alexander R.W., *Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells*, *Circ Res*, **1994**, 74:1141–1148.
91. Inagi R., Yamamoto Y., Nangaku M., Usuda N., Okamoto H., Kurokawa K., van Ypersele de Strihou C., Yamamoto H., Miyata T., *A severe diabetic nephropathy model with early development of nodule-like lesions induced by megsin overexpression in RAGE/iNOS transgenic mice*, *Diabetes*, **2006**, 55, 356–366.
92. Wendt T.M., Tanji N., Guo J., Kislinger T.R., Qu W., Lu Y., Bucciarelli L.G., Rong L.L., Moser B., Markowitz G.S., Stein G., Bierhaus A., Liliensiek B., Arnold B., Nawroth P.P., Stern D.M., D'Agati V.D., Schmidt A.M., *RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy*, *Am J Pathol*, **2003**, 162:1123–1137.
93. Flyvbjerg A., Denner L., Schrijvers B.F., Tilton R.G., Mogensen T.H., Paludan S.R., Rasch R., *Long-term renal effects of a neutralizing RAGE antibody in obese type 2 diabetic mice*, *Diabetes*, **2004**, 53(1), 166–72.
94. Tanji N., et al., *Expression of advanced glycation end products and their cellular receptor RAGE in diabetic nephropathy and nondiabetic renal disease*, *J. Am. Soc. Nephrol.*, **2000**, 11, 1656–1666.
95. Klein R., Klein B.E., Moss S.E., *Epidemiology of proliferative diabetic retinopathy*, *Diabetes Care*, **1992**, 15(12), 1875–91.
96. The Diabetes Control and Complications Trials (DCCT) Research Group. *The effect of intensive treatment of diabetes on the development and progression of longterm complications in insulin-dependent diabetes mellitus*, *N Engl J Med*, **1993**, 329, 977–986.
97. UK Prospective Diabetes Study Group. *Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33)*, *Lancet*, **1998**, 352, 837–853.
98. Kagan V.E., Shvedova A.A., Novikov K.N., Kozlov Y.P., *Lightinduced free radical oxidation of membrane lipids in photoreceptors of frog retina*, *Biochim Biophys Acta*, **1973**, 330, 76–79.
99. Anderson R.E., Rapp L.M., Wiegand R.D., *Lipid peroxidation and retinal degeneration*, *Curr Eye Res.*, **1984**, 3, 223–7.
100. Madsen-Bouterse S.A., Kowluru R.A., *Oxidative stress and diabetic retinopathy: Pathophysiological mechanisms and treatment perspectives*, *Rev Endocr Metab Disord*, **2008**, 9(4), 315–27.
101. Kowluru R.A., Chan P.S., *Oxidative Stress and Diabetic Retinopathy*, *Experimental Diabetes Research*, **2007**, 1–12.
102. Ola M.S., Berkich D.A., Xu Y., King M.T., Gardner T.W., Simpson I., LaNoue K.F., *Analysis of glucose metabolism in diabetic rat retinas*, *Am J Physiol Endocrinol Metab*, **2006**, 290, 1057–1067.
103. Kanwar M., Chan P.S., Kern T.S., Kowluru R.A., *Oxidative damage in the retinal mitochondria of diabetic mice: possible protection by superoxide dismutase*, *Invest Ophthalmol Vis Sci.*, **2007**, 48(8), 3805–11.
104. Luty G.A., McLeod D.S., Merges C., Diggs A., Plou'et J., *Localization of vascular endothelial growth factor in human retina and choroids*, *Archives of Ophthalmology*, **1996**, 114(8), 971–977.
105. Aiello L.P., Wong J.-S., *Role of vascular endothelial growth factor in diabetic vascular complications*, *Kidney International*, **2000**, 58(77), S113–S119.
106. Goh S.Y., Cooper M.E., *The Role of Advanced Glycation End Products in Progression and Complications of Diabetes*, *J Clin Endocrinol Metab*, **2008**, 93, 1143–1152.
107. Du Y., Miller C.M., Kern T.S., *Hyperglycemia increases mitochondrial superoxide in retina and retinal cells*, *Free Radic Biol Med*, **2003**, 35, 1491–1499.
108. Van den Enden M.K., Nyengaard J.R., Ostrow E., Burgan J.H., Williamson J.R., *Elevated glucose levels increase retinal glycolysis and sorbitol pathway metabolism: implications for diabetic retinopathy*, *Invest Ophthalmol Vis Sci*, **1995**, 36, 1675–1685.
109. Romeo G., Liu W.H., Asnaghi V., Kern T.S., Lorenzi M., *Activation of nuclear factor-kappaB induced by diabetes and high glucose regulates a proapoptotic program in retinal pericytes*, *Diabetes*, **2002**, 51, 2241–8.

110. Boulton A.J.M., Vinik A.I., Arezzo J.C., Bril V., Feldman E.L., Freeman R., Malik R.A., Maser R.E., Sorenko J.M., Ziegler D., *Diabetic Neuropathies*, *Diabetes Care*, **2005**, 28(4), 956–962.
111. Russel J.W., Low P., Feldman E.L., *Oxidative stress in the pathogenesis of diabetic neuropathy*, *Endocrine Reviews*, **2004**, 25(4), 612–628.
112. Pop-Bușui R., Sima A., Stevens M., *Diabetic neuropathy and oxidative stress*, *Diabetes Metab Res Rev*, **2006**, 22, 257–273.
113. Cameron N.E., Cotter M.A., *Metabolic and vascular factors in the pathogenesis of diabetic neuropathy*, *Diabetes*, **1997**, 46(Suppl. 2), 31S–37S.
114. Oates P.J., Mylari B.L., *Aldose reductase inhibitors: therapeutic implications for diabetic complications*, *Expert Opin Investig Drugs*, **1999**, 8, 2095–2119.
115. Yamagishi S., Uehara K., Otsuki S., Yagihashi S., *Differential influence of increased polyol pathway on protein kinase C expressions between endoneurial and epineurial tissues in diabetic mice*, *J Neurochem*, **2003**, 87, 497–507.
116. Srivastava A.K., *High glucose-induced activation of protein kinase signaling pathways in vascular smooth muscle cells: a potential role in the pathogenesis of vascular dysfunction in diabetes*, *Int J Mol Med*, **2002**, 9, 85–89.
117. Rosen P., Toeller M., *Vitamin E in diabetes. Increased oxidative stress and its prevention as a strategy to prevent vascular complications?* *Int J Vitam Nutr Res*, **1999**, 69, 206–212.
118. Vlassara H., Brownlee M., Cerami A., *Nonenzymatic glycosylation of peripheral nerve protein in diabetes mellitus*, *Proc Natl Acad Sci U S A*, **1981**, 78, 5190–5192.
119. Vlassara H., Brownlee M., Cerami A., *Excessive nonenzymatic glycosylation of peripheral and central nervous system myelin components in diabetic rats*, *Diabetes*, **1983**, 32, 670–674.
120. Bierhaus A., Chevion S., Chevion M., et al., *Advanced glycation end product-induced activation of NF- $\kappa$ B is suppressed by alpha-lipoic acid in cultured endothelial cells*, *Diabetes*, **1997**, 46, 1481–1490.
121. Hellweg R., Hartung H.D., *Endogenous levels of nerve growth factor (NGF) are altered in experimental diabetes mellitus: a possible role for NGF in the pathogenesis of diabetic neuropathy*, *J Neurosci Res*, **1990**, 26, 258–267.
122. Soriano F.G., Virág L., Szabó C., *Diabetic endothelial dysfunction: role of reactive oxygen and nitrogen species production and poly(ADP-ribose) polymerase activation*, *J Mol Med*, **2001**, 79, 437–448.
123. Li F., Szabo C., Pacher P., Southan G.J., Abatan O.I., Charniauskaya T., Stevens M.J., Obrosova I.G., *Evaluation of orally active poly(ADP-ribose) polymerase inhibitor in streptozotocin-diabetic rat model of early peripheral neuropathy*, *Diabetology*, **2004**, 47, 710–717.
124. Pacher P., Szabo C., *Role of Poly(ADP-Ribose) Polymerase-1 Activation in the Pathogenesis of Diabetic Complications: Endothelial Dysfunction, as a Common Underlying Theme*, *Antioxid Redox Signal.*, **2005**, 7(11–12), 1568–1580.
125. Vincent A.M., McLean L.L., Backus C., Feldman E.L., *Short term hyperglycemia produces oxidative damage and apoptosis in neurons*, *FASEB J*, **2005**, 19, 638–640.
126. Smith A.G., Singleton J.R., *Impaired glucose tolerance and neuropathy*, *Neurologist*, **2008**, 14(1), 23–29.
127. Borcea V., Nourooz-Zadeh J., Wolff S.P., et al., *alpha-Lipoic acid decreases oxidative stress even in diabetic patients with poor glycemic control and albuminuria*, *Free Radic Biol Med*, **1999**, 26, 1495–1500.
128. Haak E., Usadel K.H., Kusterer K., et al., *Effects of alpha-lipoic acid on microcirculation in patients with peripheral diabetic neuropathy*, *Exp Clin Endocrinol Diabetes*, **2000**, 108, 168–174.
129. Ziegler D., Hanefeld M., Ruhnau K.J., et al., *Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a 7-month multicenter randomized controlled trial (ALADIN III Study)*. ALADIN III Study Group. *Alpha-Lipoic Acid in Diabetic NeuroExp Clin Endocrinol Diabetespathy*, *Diabetes Care*, **1999**, 22, 1296–1301.
130. Ziegler D., Reljanovic M., Mehnert H., Gries F.A., *Alpha-lipoic acid in the treatment of diabetic polyneuropathy in Germany: current evidence from clinical trials*, *Exp Clin Endocrinol Diabetes*, **1999**, 107, 421–430.
131. Ziegler D., Schatz H., Conrad F., et al., *Effects of treatment with the antioxidant alpha-lipoic acid on cardiac autonomic neuropathy in NIDDM patients. A 4-month randomized controlled multicenter trial (DEKAN Study)*. *Diabetes Care*, **1997**, 20, 369–373.
132. Ametov A.S., Barinov A., Dyck P.J., et al., *The sensory symptoms of diabetic polyneuropathy are improved with alpha-lipoic acid: the SYDNEY trial*, *Diabetes Care*, **2003**, 26, 770–77.