ANTIOXIDANTS & REDOX SIGNALING
Volume 9, Number 3, 2007

DOI: 10.1089/ars.2006.1469

Forum Review

Renal Microvascular Injury in Diabetes: RAGE and Redox Signaling

MELINDA T. COUGHLAN, MARK E. COOPER, and JOSEPHINE M. FORBES

ABSTRACT

Diabetic nephropathy remains a major cause of morbidity and mortality in the diabetic population and is the leading cause of end-stage renal failure in the Western World. Despite current therapeutics including intensified glycemic control and blood pressure lowering agents, renal disease continues to progress relentlessly in diabetic patients, albeit at a lower rate. It is well recognized that metabolic and hemodynamic factors play a central role in accelerating renal disease in diabetes. However, recent experimental studies have suggested that increased generation of reactive oxygen species (ROS) as a result of the diabetic milieu may play a central role in the progression of diabetic microvascular complications. These ROS appear to be generated primarily from mitochondrial sources and via the enzyme, NADPH oxidase. This review focuses on how ROS play a deleterious role in the diabetic kidney and how they are involved in crosstalk among various signaling pathways, ultimately leading to renal dysfunction and structural injury. *Antioxid. Redox Signal.* 9, 331–342.

INTRODUCTION

DIABETES MELLITUS is currently at epidemic proportions in Western countries and is an emerging health problem in developing nations. Furthermore, the number of people with diabetes is predicted to double within the next 10 years (85, 140). It is the end organ injury as a result of diabetes that appears to constitute a significant proportion of mortality attributed to this condition. Individuals with diabetes suffer from an array of complications, both micro- and macrovascular. Patients with Type 1 (1) or Type 2 diabetes (2) will ultimately have diabetic complications in at least 40% of cases, even in the setting of acceptable glycemic control. As a result, a major proportion of funds allocated to the management of diabetic patients is spent in the management and treatment of its complications.

It is clearly recognized that chronic hyperglycemia is a major if not the dominant factor in the pathogenesis of the microvascular disease in diabetes (1). Microvascular pathology as a result of diabetes occurs at various sites, particularly in the retina, the renal glomerulus, and the peripheral nerve. As a consequence of this microvascular injury, diabetes is a leading cause of blindness, end stage renal disease, and neuropathy (12). People with diabetes also have an increased risk of stroke, myocardial infarction, and limb amputations due to macrovascular disease, specifically injury to arteries that supply the heart, brain, and lower limbs (12).

DIABETIC NEPHROPATHY

Diabetic nephropathy is the leading cause of renal failure in adults. The mortality of people with diabetic nephropathy is at least 10-fold higher than the general population, primarily as a result of the marked increase in cardiovascular risk that accounts for more than one-half of deaths in this population (18). Currently, diabetic kidney disease affects approximately 15–25% of patients with Type 1 diabetes (49) and up to 30–40% of Type 2 diabetic persons (96, 137).

The etiology of the structural and functional changes characteristic of diabetic nephropathy is not fully understood. The predominant structural changes seen in diabetic nephropathy are extracellular matrix accumulation of proteins such as collagen, laminin, and fibronectin, which lead

Albert Einstein Centre for Diabetes Complications, Wynn Domain, Baker Heart Research Institute, Melbourne, Victoria, Australia.

to mesangial expansion and glomerular basement membrane thickening, in addition to tubulointerstitial fibrosis (64, 100). The nature of these structural alterations varies, with diabetic nephropathy considered a heterogeneous mixture of various renal pathologies that are induced and sustained by different mechanisms and that may coexist in different combinations (100). Pure diabetic glomerulopathy is more frequently observed in patients with earlier onset of diabetes and commonly seen already at the stage of microalbuminuria (9). By contrast, less specific vascular and tubulo-interstitial changes are more prominent in older patients with macroalbuminuria, renal insufficiency, and a long history of arterial hypertension (35).

ETIOLOGY OF MICROVASCULAR DISEASE

It remains to be determined if there is a common factor underlying the disturbances in the microvasculature in diabetes. It has been established that hyperglycemia is the likely initiating factor in tissue damage observed clinically in diabetes (1, 2). However, only particular cell types are damaged in diabetes, such as mesangial cells within the glomerulus, capillary endothelial cells in the retina, and neurons and Schwann cells in the peripheral nerves (13). The vulnerability of these cells to high glucose-induced damage is distinct from other cell types such as adipocytes, myocytes, and hepatocytes. This is most likely due to the ability of these cells, which are resistant to the adverse effects of high glucose, to negatively regulate the entry of glucose into the cell. Glucose transport into mesangial and some endothelial cells (dependent on vascular bed) occurs by a facilitated diffusion process, which is independent of insulin action and occurs at an accelerated rate during hyperglycemia (45, 60). This excess in intracellular glucose bioavailability triggering an increase in glucose utilization has been postulated to initiate various pathways of hyperglycemic tissue damage (13).

Indeed, according to Brownlee's "unifying hypothesis" (12), hyperglycemia-induced damage in diabetic complications is due to mitochondrial superoxide overproduction that then activates four major biochemical pathways. These include increased advanced glycation end product (AGE) formation, activation of protein kinase C (PKC) isoforms, increased polyol pathway flux, and increased hexosamine pathway flux (Fig. 1). Continued research into these pathways has confirmed the significance of increased reactive oxygen species (ROS) formation and depletion in antioxidant defence in the development of diabetic complications.

INCREASED POLYOL PATHWAY FLUX

The first hyperglycemia-induced pathway of damage that was documented 40 years ago (34) is increased flux through the polyol pathway. The cytosolic enzyme aldose reductase reduces high intracellular glucose to sorbitol using NAD(P)H as a cofactor. Subsequently, sorbitol is oxidized to fructose via sorbitol dehydrogenase, with NAD^+ reduced to NADH. Sorbitol does not cross cell membranes, but accumulates intracellularly with resultant osmotic stress (59, 69). Increased sorbitol production generated by the polyol pathway in the

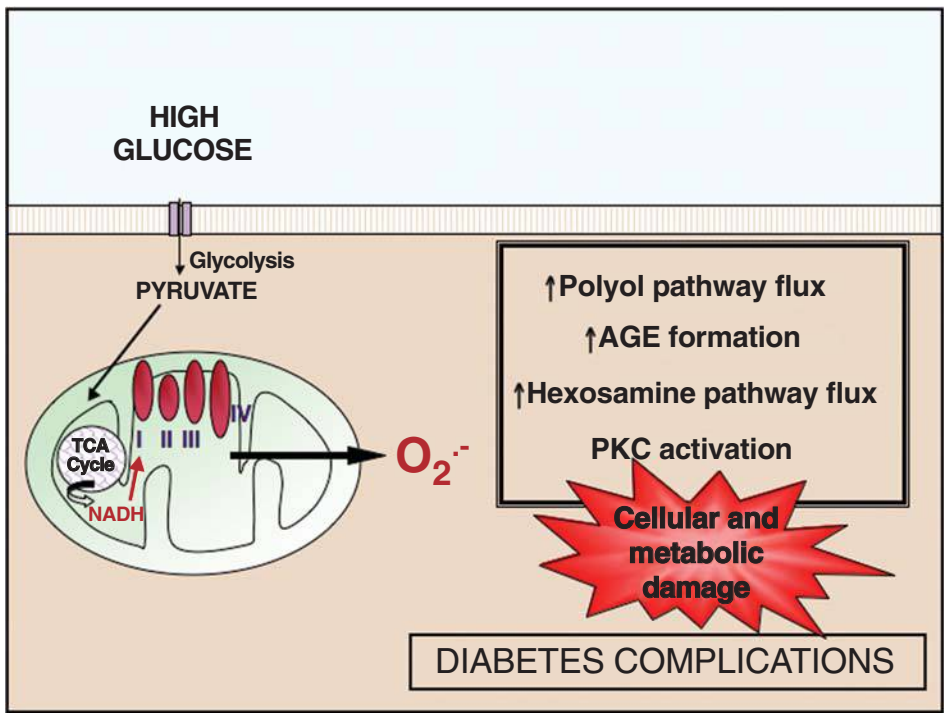

FIG. 1. The "Brownlee hypothesis." Superoxide-mediated activation of four major pathways that lead to diabetes complications.

presence of elevated glucose concentrations results in an intracellular depletion of NAD(P)H. The intracellular depletion of NAD(P)H inhibits regeneration of reduced glutathione, a critical antioxidant. This depletion in glutathione may increase susceptibility to intracellular oxidative stress (70). Indirect biochemical consequences of increased sorbitol pathway activity include nonenzymatic glycation initiated by fructose, which is 10 times more potent as a glycating agent than glucose (113), activation of PKC, oxidative and nitrosative stress, and oxidative stress-mediated downstream events, including activation of mitogen-activated protein kinases (MAPKs) and poly(ADP-ribose) polymerase (PARP) (89). The significance of the polyol pathway in diabetes pathophysiology has been observed in diabetic animal models treated with aldose reductase inhibitors. Aldose reductase inhibition has been shown to delay, prevent, and at early stages, to reverse diabetic complications (89). Clinical trials with two aldose reductase inhibitors, zenarestat and fidarestat, have demonstrated that inhibition of aldose reductase in the nerve improved both nerve physiology and fiber density as well as function (37, 48). However, the clinical role of aldose reductase inhibitors remains uncertain despite decades of investigation and has been marred by limited tissue penetration and a range of side effects.

INCREASED HEXOSAMINE PATHWAY FLUX

When the glucose concentration within the cell is in excess, an additional route of glucose metabolism is enhanced, the hexosamine biosynthetic pathway. Glucose-6-phosphate is converted to fructose-6-phosphate, which, in the presence of a normal glucose environment, is metabolized via glycolysis. However, during conditions of high intracellular glucose, the enzyme glutamine:fructose-6 phosphate amidotransferase (GFAT) can convert fructose-6-phosphate to glucosamine-6-phosphate and subsequently to UDP (uridine diphosphate) *N*-acetyl glucosamine. Intracellular glycosylation by the addition of *N*-acetyl glucosamine (GlcNAc) to serine and threonine is catalyzed by the enzyme *O*-GlcNAc transferase. These moieties can bind to transcription factors such as Sp-1 and increase the synthesis of factors such as TGF-β1 and PAI-1, both of which are detrimental to blood vessels (25). This enhanced flux via the hexosamine pathway generating toxic metabolites is another mechanism by which hyperglycemia can induce cell damage. The role of this pathway remains to be fully delineated and the clinical applicability of these findings has been limited by a lack of orally active specific agents to target this pathway.

ACTIVATION OF PROTEIN KINASE C

An alternate, well-studied mechanism whereby increased intracellular glucose confers damage in the microvasculature is the protein kinase C (PKC) pathway. High glucose can stimulate the lipid second messenger diacylglycerol (DAG) through the glycolytic intermediate dihydroxyacetone phosphate (68). DAG can activate the classic isoforms of PKC, α, β, and δ (23, 67, 68, 131). Stimulation of PKC results in a variety of changes in gene expression, which have important consequences for the diabetic state. First, PKC induces proinflammatory gene expression through the transcription factor nuclear factor-κB (NF-κB) (5, 24). Second, PKC can stimulate ROS production through NAD(P)H oxidase activation (29, 52). Furthermore, activation of PKC by glucose in mesangial cells alters prostaglandin production and induces overexpression of TGF-β1 and various extracellular matrix components (67). Hyperglycemia may also activate PKC isoforms indirectly via binding to AGE receptors (discussed later). The importance of this pathway has been clearly demonstrated using PKC inhibitors such as ruboxistaurin. Indeed, inhibition of PKC in animal models has prevented renal and retinal dysfunction (27, 54, 66). In a study from our laboratory, streptozotocin-induced diabetic rats were treated with an inhibitor of AGE accumulation, ALT-711. Diabetes-induced increases in PKC-α, -βI, -βII, and -ε isoforms were abrogated with ALT-711 in association with reduced renal AGE accumulation. Translocation of phosphorylated PKC-α from the cytoplasm to the membrane was reduced by ALT-711. ALT-711 treatment attenuated expression of vascular endothelial growth factor and the extracellular matrix proteins, fibronectin and laminin, in association with reduced albuminuria. These findings implicate AGEs as important stimuli for the activation of PKC, particularly PKC-α, in the diabetic kidney (118).

INCREASED ADVANCED GLYCATION END PRODUCT FORMATION

Hyperglycemia accelerates the formation and accumulation of AGEs (14). When glucose and other reactive carbonyl compounds react nonenzymatically with proteins, lipids, or nucleic acids, Schiff bases and Amadori products are formed. These early glycation products undergo further modification and rearrangement to generate nonreversible AGEs. Among the many potential pathogenic factors responsible for the development of diabetic microvascular disease, the advanced glycation pathway is thought to be a pivotal process in mediating tissue damage. Clinical studies in patients with Type 1 diabetes demonstrate a strong correlation between AGE accumulation and the severity of micro- and macrovascular complications (77, 81, 105, 128). In particular, serum concentrations of AGEs are significantly increased with the progression to microalbuminuria and subsequently to overt nephropathy (77). Similar results have been demonstrated correlating skin collagen-associated levels of AGEs with the severity of complications in patients with long-standing Type 1 diabetes (81) and with carotid intimal thickening, a marker of macrovascular disease (86). In individuals with Type 2 diabetes, the circulating AGE concentration is increased, is an independent determinant of plasma C-reactive protein levels (116), and correlates with hypertension and ischemic heart disease in this population (114).

In addition to the circulation, AGEs have been found at increased concentrations in various sites of injury in diabetes.

AGE accumulation has been observed in renal glomeruli (47) and tubules (30), retinal vessels (112), and in peripheral nerve components (76). Indeed, a study of pharmacological inhibition of AGEs over a decade ago using the relatively nonspecific AGE formation inhibitor, aminoguanidine, showed amelioration of both structural and functional features of experimental diabetic nephropathy (111). Since then, numerous experimental studies have supported and extended these findings (56). Within diabetic tissues, AGEs are thought to contribute to end organ injury via a number of processes, such as by inducing cross-linking of proteins and through ligand-receptor binding (Fig. 2).

AGE-INDUCED STRUCTURAL CHANGES

AGEs were initially demonstrated to promote permanent structural alterations in the extracellular matrix, in particular, by inducing cross-linking of proteins (15). Some of the best-characterized AGEs, such as pentosidine, methylglyoxal lysine dimer, and glyoxal lysine dimer, represent intermolecular cross-links between modified proteins (120). These cross-links can result in important changes to protein structure and function. A good example is the formation of inter- and intramolecular cross-links, following the glycation of collagen, which lead to structural alterations, including changes in packing density (7) and surface charge (42), manifested by increased stiffness, reduced thermal stability, and resistance to proteolytic digestion (83, 108).

INTRACELLULAR AGE FORMATION

Elevated intracellular glucose degradation products resulting from glycolysis and the TCA cycle initiate the glycation of proteins far more rapidly than glucose itself (43). Therefore, the enhanced glycation of proteins observed in diabetic tissues may be partly attributable not to a high concentration of glucose itself, but increased levels of these intermediate "highly reactive" metabolites induced by hyperglycemia. AGEs can be generated from intracellular autoxidation of glucose to glyoxal (127), decomposition of the Amadori product to 3-deoxyglucosone, and fragmentation of glyceraldehyde-3-phospate and dihydroxyacetone phosphate to methylglyoxal (119).

In bovine aortic endothelial cells, hyperglycemia generates intracellular AGEs via the AGE-intermediate, methylglyoxal (106), a precursor to a well-characterized AGE, *N*ε-carboxymethyl)lysine (CML). This process has been subsequently localized to the mitochondria in a study that showed that high glucose-induced intracellular formation of methylglyoxal-derived AGEs was completely prevented by inhibitors of mitochondrial complex II and III, uncoupling protein I and manganese-containing superoxide dismutase (MnSOD or SOD2) (88). We have recently found that intracellular formation of CML within the renal mitochondria of rats with streptozotocin-induced diabetes was increased twofold compared to nondiabetic rats (20). This formation seemed to be glucose-dependent, as AGEs (AGE-RSA) given exogenously to healthy rats daily did not induce increased mi-

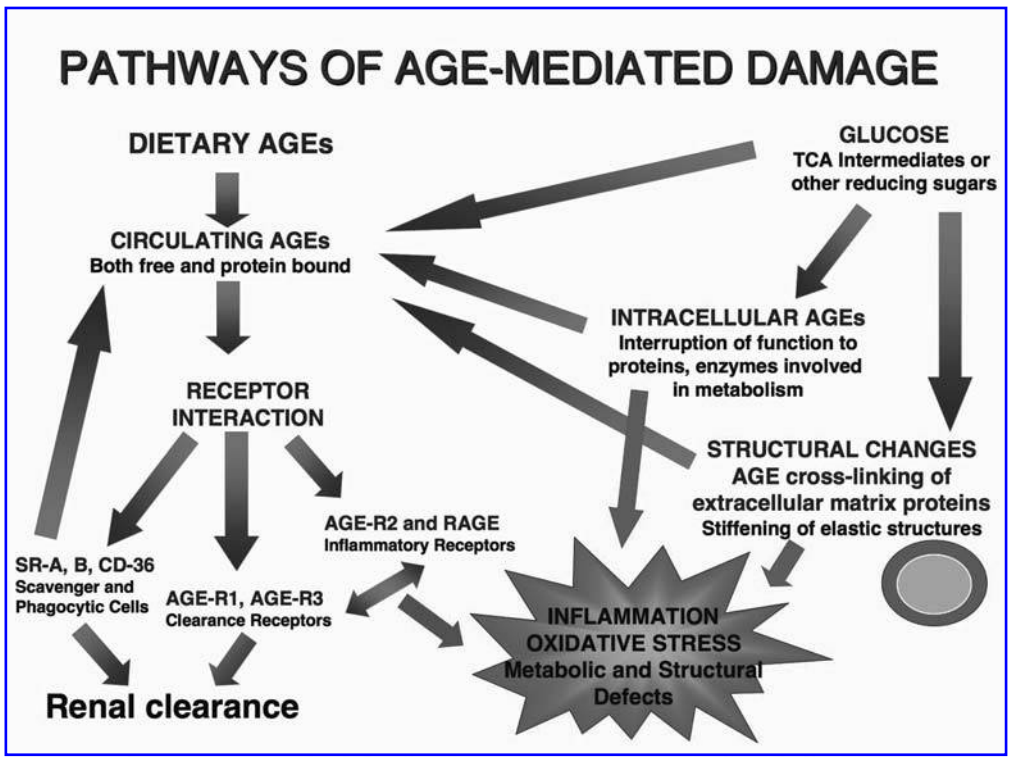

FIG. 2. Pathways of AGE-mediated damage.

tochondrial formation of CML in the face of normoglycemia (31).

The generation of intracellular AGEs can disturb redox homeostasis by modifying protein and enzyme structure and function. For example, glycation of antioxidants such as copper and zinc-containing SOD (CuZn-SOD or SOD1) contribute to the decline in antioxidant activity (33). Whereas oxidative stress can augment the formation of AGEs through glycoxidation, AGEs can also lead to enhanced formation of free radicals, both directly through catalytic sites in their molecular structure (132) and via stimulation of membrane-bound NAD(P)H oxidase through the RAGE receptor (126).

AGE RECEPTOR-MEDIATED EFFECTS

AGEs can also act directly as effector molecules by inducing receptor-mediated changes in cell transduction and modulating a range of signaling proteins (104, 123). AGEs appear to mediate their effects via interactions with specific receptors and binding proteins. These receptors are present on various renal cell types including proximal tubular cells, mesangial cells, and podocytes (110, 130). Several AGE-binding sites have been identified. These include the receptor for advanced glycation end products (RAGE), AGE-R1 (p60), AGE-R2 (80k-H, protein kinase C substrate), AGE-R3 (galectin-3), lysozyme (122), as well as the macrophage scavenger receptors ScR-II and CD-36 (109) and the more recently identified members of the ezrin–radixin–moesin family (75). Other multiligand receptors such as megalin may also have the ability to bind AGEs in the proximal tubule (101). Most of these AGE-binding proteins are constitutively expressed in a limited number of cell types at low levels in the absence of injury and inflammation. Expression of some of these receptors is markedly enhanced in response to metabolic states such as diabetes, dyslipidemia, and uremia, possibly due to high levels of AGEs in these conditions. In particular, activated cells at sites of diabetes-associated injury show high level expression of receptors such as RAGE and colocalize with AGE deposition (110).

AGEs can interact with inflammatory, oxidative, metabolic, and hemodynamic pathways. These diverse interactions appear to be predominantly related to signaling through the multiligand AGE receptor, RAGE. RAGE is a member of the immunoglobulin superfamily of cell surface molecules (87, 103) and is the best characterized signal transduction receptor for AGEs. RAGE is expressed by a number of cells whose function is perturbed in diabetes, with the expression of this receptor increased at sites of vascular pathology in diabetes (103). There is an increasing body of evidence to support the concept that interactions between AGEs and their receptors, especially RAGE, are involved in the pathogenesis of diabetic complications, in particular, nephropathy (14, 32, 111). For example, studies in RAGE transgenic mice reveal that these rodents have increased glomerulosclerosis following the induction of diabetes (133). Conversely, RAGE knockout mice have decreased renal injury in response to diabetes (130) and long-term administration of a RAGE neutralizing antibody to *db/db*$^{(+/+)}$ mice confers renoprotection (28). In the glomeruli of patients with diabetic nephropathy, RAGE expression is upregulated and positively correlates with AGE accumulation (117). These studies underscore the significance of AGE-receptor-mediated pathways in the pathogenesis of diabetic nephropathy.

RAGE has a central role in mediating the effects of AGEs on the development of vascular disease in diabetes (46). Engagement of RAGE by its ligands induces inflammatory cell infiltration and activation in the vessel wall. In diabetes, the AGE–RAGE axis amplifies vascular stress and accelerates atherosclerosis and neointimal expansion (84).

A key consequence of the interaction of AGEs with RAGE is the generation of ROS (78, 102, 103, 124, 125, 134). The AGE–RAGE interaction has been shown to result in the generation of thiobarbituric acid reactive substances (TBARS), increased mRNA for heme oxygenase-1, increased endothelial expression of vascular cell adhesion molecule-1 (VCAM-1), and promotion of endothelial permeability (102, 103, 124). These studies suggest that generation of ROS with subsequent increased oxidant stress is a potent factor initiating signal transduction and altered gene expression, as a result of the AGE–RAGE interaction. Indeed, the effects of this ligand–receptor interaction were inhibited by antioxidants such as *N*-acetylcysteine, probucol, or vitamin E (102, 124, 125).

RAGE-mediated signaling appears to augment inflammation and enhance tissue damage. Nuclear factor-κB is a redox sensitive transcription factor that is critically involved in inflammatory processes. The RAGE promoter contains NF-κB binding sites that are active and are involved in the regulation of RAGE expression (71). Engagement of RAGE by AGEs results in the generation of ROS and subsequent activation of NF-κB (134). AGE receptors trigger the activation of the JAK/STAT signal transcription pathway (135), leading to the upregulation of transcription factors and intracellular signaling molecules such as PKC (92) and increased production of inflammatory and fibrogenic growth factors and cytokines including TGF-β (62), CTGF (121), PDGF (62), TNF-α, IL-1β, and IL-6 (79). These effects appear to be predominantly receptor mediated, as specific antibodies to AGE-receptors are able to block these changes without reducing AGE levels (51). Adhesion molecules such as VCAM-1 and intercellular adhesion molecule (ICAM-1) are also induced by AGEs, although it still remains controversial as to whether this is a result of activation of RAGE and subsequent NF-κB activation (11).

Recently, it has been demonstrated that there are three splice variants of RAGE. There is a full length receptor, the N-terminal variant that does not contain the AGE-binding domain and the C-terminal splice variant, soluble RAGE (sRAGE), which does not contain the transmembrane and effector domains (74, 138). sRAGE, comprising the extracellular portion of the RAGE receptor, has the ability to bind AGEs and thereby block interactions with full-length cell surface RAGE (125). It has been postulated that this effect of sRAGE may provide therapeutic benefits for diabetic nephropathy (130). Indeed, exogenous administration of sRAGE, has demonstrated beneficial effects in diabetes-associated atherosclerosis (93) and nephropathy (28, 129, 130).

OXIDATIVE STRESS AND DIABETES COMPLICATIONS

The "unifying hypothesis" described by Brownlee (discussed earlier) suggests that the generation of mitochondrial ROS is the primary initiating event in activating a number of other pathways implicated in the development of the complications of diabetes (88). There remains debate, however, as to whether oxidative stress is an important early link between hyperglycemia and complications, or just a byproduct of various pathogenic mechanisms (8).

The significance of ROS in the pathogenesis of diabetic nephropathy is further emphasized by a recent study in which glucose-induced ROS production initiates podocyte apoptosis leading to podocyte depletion with onset of hyperglycemia in Akita mice with Type 1 diabetes and db/db mice with obesity and Type 2 diabetes (115).

Because of the ability of ROS to directly oxidize and damage DNA, protein, and lipids, it is believed that these species play a key direct role in the development of late diabetic complications (12, 98). In addition to the ability to directly inflict macromolecular damage, ROS function as signaling molecules and can induce a number of stress-sensitive pathways that cause cellular damage. There are a number of sources for the generation of ROS in diabetes including autoxidation of glucose, transition metal catalyzed Fenton reactions, mitochondrial respiratory chain deficiencies, xanthine oxidase activity, and activation of microsomal enzymes, arachidonic acid, peroxidases, NO synthase, and NAD(P)H oxidase (8, 17). The discussion within this article will be restricted to only two sources of ROS, mitochondrial ROS generation and NAD(P)H oxidase. This is not, however, to relegate the importance of these other pathways that lead to ROS. Indeed, xanthine oxidase activity is inhibited in the presence of the AGE formation inhibitor, aminoguanidine (21), while Cu^{2+} augmentation of ROS-mediated AGE formation is suggested *in vitro* (91).

MITOCHONDRIAL PRODUCTION OF ROS

Reactive oxygen species are generated within the mitochondria as a result of electron flux through the electron transport chain. Pyruvate derived from glycolysis is transported into the mitochondria, where it is oxidized by the tricarboxylic acid (TCA) cycle to produce NADH and reduced flavin adenine dinucleotide ($FADH_2$). Electron flow through the mitochondrial electron transport chain is carried out by four inner membrane-associated enzyme complexes, plus cytochrome c and the mobile carrier coenzyme Q. NADH derived from the TCA cycle donates electrons to Complex I (NADH:ubiquinone oxidoreductase). Complex I ultimately transfers its electrons to coenzyme Q. Coenzyme Q is also reduced by electrons donated from several $FADH_2$-containing dehydrogenases, such as the TCA cycle succinate:ubiquinone oxidoreductase (Complex II). Electrons from reduced coenzyme Q are then transferred to Complex III. Electron transport then proceeds through cytochrome c, Complex IV, and, finally, molecular oxygen. Electron transfer through Complexes I, III, and IV generates a proton gradient. Much of the energy of this voltage gradient is used to generate ATP during oxidative phosphorylation (OXPHOS). The collapse of the proton gradient through ATP synthase drives ATP synthesis. This energy can also be dissipated as heat via uncoupling proteins (UCPs).

During OXPHOS, a low proportion of molecular oxygen is converted to superoxide and subsequently hydrogen peroxide and the hydroxyl radical, which, under normal conditions, are scavenged by antioxidant enzymes, including mitochondrial MnSOD (SOD2) or glutathione peroxidase (GPx). Damaged or dysfunctional mitochondria, however, overgenerate superoxide radicals, creating a state of redox imbalance (94). Excess mitochondrial ROS production is often mediated by disruption of the activity of OXPHOS enzymes via electron leakage at complex I (NADH:ubiquinone oxidoreductase) or complex III (ubiquinol:cytochrome c oxidoreductase) (10, 65, 94). Indeed, in Freidreich ataxia, a genetic disorder due to frataxin mutations resulting in excessive generation of mitochondrial superoxide, mitochondria have a specific deficiency in complex I (99).

Mitochondrial electron transport chain dysfunction has also been implicated in the generation of reactive nitrogen species (RNS). Nitric oxide can react with superoxide to form the highly reactive peroxynitrite ($ONOO^-$) that can traverse membranes and react with tyrosine residues forming 3-nitrotyrosine (44). Peroxynitrite can irreversibly inhibit cytochrome *c* oxidase (complex IV), leading to further generation of superoxide and peroxynitrite, resulting in further damage to the electron transport chain (44).

A recent study has found that renal cortical mitochondria from rats with diabetes exhibited a diminution of OXPHOS via decreased complex III activity and increased superoxide formation (97). Complex III activity correlated with the quantity of methylglyoxal-induced modifications present on mitochondrial proteins. Our own group has demonstrated overproduction of superoxide from renal mitochondria in rats with diabetic nephropathy in parallel with increased intramitochondrial CML accumulation and deficiencies in complex I and MnSOD activity (20).

Intramitochondrial superoxide production initiates a range of damaging reactions through the production of hydrogen peroxide, ferrous iron, hydroxyl radical, and peroxynitrite, which can damage lipids, proteins, and nucleic acids. The normal function of mitochondria is particularly susceptible to ROS damage, leading to altered ATP synthesis, cellular calcium dysregulation, and induction of mitochondrial permeability transition, all of which predispose the cell to necrosis or apoptosis (55).

THE NAD(P)H OXIDASE AND ROS PRODUCTION

The mitochondrion is not the sole source of oxidative stress within the cell. NAD(P)H oxidase is a cytosolic enzyme complex that was initially discovered in neutrophils where it plays a vital role in nonspecific host defense by producing millimolar quantities of superoxide (6). The enzyme

complex is made up of five subunits comprising a membrane-associated cytochrome b_{558}, composed of one $p22^{phox}$ and one $gp91^{phox}$ subunit and at least four cytosolic subunits: $p47^{phox}$, $p67^{phox}$, $p40^{phox}$, and GTPase *rac*1 or *rac*2 (6). In addition to residing in phagocytic cells, NAD(P)H oxidase is present in nonphagocytic cell types such as renal mesangial and tubular cells, vascular smooth muscle cells, endothelial cells, and fibroblasts (38, 58, 95, 107, 136, 139). In these cell types, however, superoxide is produced constitutively at low levels and when the NAD(P)H oxidase activity is upregulated, detectable superoxide production is proportionally lower than in activated neutrophils. The function of NAD(P)H oxidase in nonphagocytic cells is clearly different to that seen in white blood cells, as ROS are generated in this context in the intracellular compartment as opposed to outside the cell during phagocytosis. This has led to the suggestion that small amounts of ROS, generated by nonphagocytic NAD(P)H oxidase, may participate in second messenger redox signaling, and when upregulated, greater ROS production contributes to oxidative stress (38).

That NAD(P)H oxidase participates in redox signaling is evident from studies demonstrating that ROS generated by NAD(P)H oxidase are involved signal transduction in response to several cytokines such as TNF-α, PDGF, and angiotensin II (39, 72). Binding of these cytokines to their cognate receptors rapidly activates NAD(P)H oxidase followed by intracellular superoxide and hydrogen peroxide generation and activation of signaling molecules, including protein tyrosine kinases, serine/threonine kinases, phospholipases, and calcium-dependent pathways (72). The evidence that specific NAD(P)H oxidase-derived ROS production is required for activation of these pathways has been obtained from studies using pharmacological inhibition of NAD(P)H oxidase, mice with deletions of the various NAD(P)H oxidase subunits, or treatment with antisense oligonucleotides (72).

In addition to regulating typical redox signaling pathways, nonphagocytic NAD(P)H oxidase can also lead to excessive ROS production, culminating in oxidative stress. This has been shown in pathological states such as atherosclerosis, hypertension, inflammation, and ischemia–reperfusion injury (38, 139). The expression of NAD(P)H oxidase components is increased in micro- and macrovascular tissues of diabetic animals. Incubation of human endothelial cells with AGEs including the specific AGE, CML, on the surface of diabetic red blood cells, induced intracellular generation of hydrogen peroxide, cell surface expression of VCAM-1, and generation of tissue factor, which was suppressed by treatment with the NAD(P)H oxidase inhibitor diphenyliodonium (126). This appears to be clinically relevant with endothelial dysfunction, linked to NAD(P)H oxidase ROS generation having been observed in animal models, as well as in patients with diabetes (3, 40).

In the kidney, various subunits of the NAD(P)H oxidase are increased in rats with diabetes (4, 26, 53, 63, 90). Studies from our own laboratory have demonstrated increases in AGEs and nitrotyrosine in association with the expression of RAGE, the NAD(P)H oxidase subunit $gp91^{phox}$ and NF-κB in the kidney of rats with diabetes (30). *In vitro,* high glucose activates NAD(P)H oxidase subunits in mesangial cells, perhaps via PKC (41, 50, 53). Furthermore, pharmacological inhibition of NAD(P)H oxidase with apocynin prevents $p47^{phox}$ and $gp91^{phox}$ overexpression and retards the mesangial matrix expansion in rats with diabetic nephropathy (4). A more specific approach has been applied involving treatment of diabetic rats with antisense Nox-4 (the renal $gp91^{phox}$ homologue) oligonucleotide treatment. This strategy inhibited NAD(P)H-dependent ROS generation in renal cortical and glomerular homogenates and reduced whole kidney and glomerular hypertrophy (36). These data highlight the importance of the NAD(P)H oxidase in high glucose-induced ROS production. Indeed, preliminary data from our laboratory has observed NAD(P)H oxidase-driven ROS formation in the renal cortex from rats with diabetic nephropathy (unpublished observations). Furthermore, a recent study has demonstrated that in mesangial cells *in vitro*, NAD(P)H oxidase was responsible for high glucose- and AGE-induced superoxide production, which mediated elevations in TGF-β1 and fibronectin levels (73).

THERAPEUTIC APPROACHES TO TARGET DYSFUNCTION

Numerous studies have demonstrated that oxidative stress, mediated predominantly by hyperglycemia-induced generation of ROS, contributes to the development of microvascular complications of diabetes. Even though studies using antioxidants in experimental models of diabetes indicate that antioxidants should confer beneficial effects in reducing microvascular complications in diabetes, the clinical evidence for the use of antioxidants in this setting is not yet established. Positive effects of antioxidant therapy in experimental diabetic nephropathy have been described. For example, supplementation of streptozotocin-induced diabetic rats with vitamins C and E decreased urinary albumin excretion, glomerular basement membrane thickening, and kidney weight. This occurs in the context of decreased TBARS and an increase in SOD and catalase activity (61). There are many conflicting studies, however, in projects using nutrient derived antioxidants (recently reviewed in Ref. 57). Clinical trials with conventional antioxidants have similarly yielded conflicting data.

It has been suggested that the antioxidant efficacy of conventional vitamins such as Vitamin C and E is limited because these antioxidants work as scavengers of existing excess ROS in a stoichiometric manner (12). There is a need for the development of methods for selectively delivering biologically active molecules to the site of the mitochondrial electron transport chain where excess superoxide generation can be dampened. New low molecular mass compounds that act as SOD or catalase mimetics have the theoretical advantage of scavenging ROS continuously by acting as catalysts with efficiencies approaching those of the native antioxidant enzymes (22). Other agents already used in clinical practice have been found to have ROS scavenging properties. Thiazolidinediones, statins, ACE, and AT1 inhibitors have intracellular antioxidant activity and it has been suggested that many of their ancillary effects are due to this property (19). Indeed, in a study by our group, in a model of diabetes-associated atherosclerosis, the PPAR-alpha agonist conferred anti-atherosclerotic effects in the setting of decreased superoxide pro-

duction and decreased expression of various subunits of NAD(P)H oxidase (16).

There are several currently identified processes by which AGE inhibition is achieved. Direct chemical inhibition of the formation of AGEs has been demonstrated. The process of dicarbonyl scavenging involves the trapping of reactive carbonyl and dicarbonyl compounds (RCOs), the critical precursors of AGEs. Entrapment of RCOs, such as glyoxal and methylglyoxal, inhibit AGE formation, as shown by several compounds, including aminoguanidine, pyridoxamine, and OPB-9195 (80). Another pathway of AGE inhibition is via blockade of ROS. Oxidative metabolism is important in the generation of RCOs (82), its inhibition subsequently reduces the formation of AGEs. Both olmesartan and emocaprilat have displayed free radical scavenging properties, decreasing the production of hydroxyl radicals and carbon-centered radicals (80). Chelation of transition metal ions also attenuates the formation of AGEs. Olmesartan and OPB-9195 (an AGE inhibitor) chelate copper ions and inhibit the autoxidation of ascorbic acid to a greater extent than aminoguanidine (80). Other compounds cleave AGE cross-links or reverse or block interaction with RAGE. A promising new therapy for diabetic nephropathy may be treatment with sRAGE (130). Indeed, exogenous administration of sRAGE, has demonstrated beneficial effects in diabetes-associated atherosclerosis (93) and nephropathy (28, 130).

CONCLUSIONS

It is clear that oxidative stress plays an important role in the progression of diabetic complications including nephropathy. The exact source of the increased ROS remains to be fully determined and the relative importance of antioxidant defense in the kidney has not been adequately examined. Nevertheless, with increasing elucidation of oxidative stress pathways and how they interact with pathways such as advanced glycation should lead to new targets and hopefully new treatments for this major complication of diabetes.

ABBREVIATIONS

AER, albumin excretion rate; AGE, advanced glycation end product; ALA, alagebrium; CML, *N*ε(carboxymethyl)lysine; DAG, diacylglycerol; GFAT, glutamine:fructose-6 phosphate amidotransferase; Gpx, glutathione peroxidase; GlcNAc, *N*-acetyl glucosamine; H_2O_2, hydrogen peroxide; ICAM, intercellular adhesion molecule; MAPKs, mitogen-activated protein kinases; NF-κB, nuclear factor-κB; OXPHOS, oxidative phosphorylation; $O_2^{\bullet-}$, superoxide radical; PKC, protein kinase C; PARP, poly(ADP-ribose) polymerase; RAGE, receptor for advanced glycation end products; RCOs, dicarbonyl compounds; ROS, reactive oxygen species; SOD, superoxide dismutase; sRAGE, soluble RAGE; TCA, tricarboxylic acid; UCP, uncoupling protein; VACM, vascular cell adhesion molecule.

REFERENCES

1. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med* 329: 977–986, 1993.
2. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with Type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 352: 837–853, 1998.
3. Ammar RF Jr, Gutterman DD, Brooks LA, and Dellsperger KC. Free radicals mediate endothelial dysfunction of coronary arterioles in diabetes. *Cardiovasc Res* 47: 595–601, 2000.
4. Asaba K, Tojo A, Onozato ML, Goto A, Quinn MT, Fujita T, and Wilcox CS. Effects of NADPH oxidase inhibitor in diabetic nephropathy. *Kidney Int* 67: 1890–1898, 2005.
5. Asehnoune K, Strassheim D, Mitra S, Yeol Kim J, and Abraham E. Involvement of PKCalpha/beta in TLR4 and TLR2 dependent activation of NF-kappaB. *Cell Signal* 17: 385–394, 2005.
6. Babior BM, Lambeth JD, and Nauseef W. The neutrophil NADPH oxidase. *Arch Biochem Biophys* 397: 342–344, 2002.
7. Bai P, Phua K, Hardt T, Cernadas M, and Brodsky B. Glycation alters collagen fibril organization. *Connect Tissue Res* 28: 1–12, 1992.
8. Baynes JW and Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* 48: 1–9, 1999.
9. Bertani T, Gambara V, and Remuzzi G. Structural basis of diabetic nephropathy in microalbuminuric NIDDM patients: a light microscopy study. *Diabetologia* 39: 1625–1628, 1996.
10. Beyer RE. An analysis of the role of coenzyme Q in free radical generation and as an antioxidant. *Biochem Cell Biol* 70: 390–403, 1992.
11. Bierhaus A, Schiekofer S, Schwaninger M, Andrassy M, Humpert PM, Chen J, Hong M, Luther T, Henle T, Kloting I, Morcos M, Hofmann M, Tritschler H, Weigle B, Kasper M, Smith M, Perry G, Schmidt AM, Stern DM, Haring HU, Schleicher E, and Nawroth PP. Diabetes-associated sustained activation of the transcription factor nuclear factor-kappaB. *Diabetes* 50: 2792–2808, 2001.
12. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 414: 813–820, 2001.
13. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 54: 1615–1625, 2005.
14. Brownlee M, Cerami A, and Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 318: 1315–1321, 1988.
15. Brownlee M, Vlassara H, and Cerami A. Nonenzymatic glycosylation and the pathogenesis of diabetic complications. *Ann Intern Med* 101: 527–537, 1984.
16. Calkin AC, Cooper ME, Jandeleit–Dahm KA, and Allen TJ. Gemfibrozil decreases atherosclerosis in experimental diabetes in association with a reduction in oxidative stress and inflammation. *Diabetologia* 49: 766–774, 2006.
17. Cameron NE and Cotter MA. Effects of antioxidants on nerve and vascular dysfunction in experimental diabetes. *Diabetes Res Clin Pract* 45: 137–146, 1999.
18. Caramori ML and Mauer M. Diabetes and nephropathy. *Curr Opin Nephrol Hypertens* 12: 273–282, 2003.
19. Ceriello A. New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. *Diabetes Care* 26: 1589–1596, 2003.
20. Coughlan MT, Thorburn DR, Fukami K, Laskowski A, Thallas–Bonke V, Long DM, Brownlee M, Cooper ME, and Forbes JM. Renal intra-mitochondrial glycation drives deficiencies in the activity of manganese superoxide dismutase and complex I of the mitochondrial respiratory chain in diabetes. *Diabetologia* 48 Suppl. 1 (Abstract): A90, 2005.
21. Courderot–Masuyer C, Dalloz F, Maupoil V, and Rochette L. Antioxidant properties of aminoguanidine. *Fundam Clin Pharmacol* 13: 535–540, 1999.

22. Cuzzocrea S, Riley DP, Caputi AP, and Salvemini D. Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. *Pharmacol Rev* 53: 135–159, 2001.
23. Derubertis FR and Craven PA. Activation of protein kinase C in glomerular cells in diabetes. Mechanisms and potential links to the pathogenesis of diabetic glomerulopathy. *Diabetes* 43: 1–8, 1994.
24. Devaraj S, Venugopal SK, Singh U, and Jialal I. Hyperglycemia induces monocytic release of interleukin-6 via induction of protein kinase c-{alpha} and -{beta}. *Diabetes* 54: 85–91, 2005.
25. Du XL, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, Wu J, and Brownlee M. 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* 97: 12222–12226, 2000.
26. Etoh T, Inoguchi T, Kakimoto M, Sonoda N, Kobayashi K, Kuroda J, Sumimoto H, and Nawata H. Increased expression of NAD(P)H oxidase subunits, NOX4 and p22phox, in the kidney of streptozotocin-induced diabetic rats and its reversibity by interventive insulin treatment. *Diabetologia* 46: 1428–1437, 2003.
27. Feener EP, Xia P, Inoguchi T, Shiba T, Kunisaki M, and King GL. Role of protein kinase C in glucose- and angiotensin II-induced plasminogen activator inhibitor expression. *Contrib Nephrol* 118: 180–187, 1996.
28. Flyvbjerg A, Denner L, Schrijvers BF, Tilton RG, Mogensen TH, Paludan SR, and Rasch R. Long-term renal effects of a neutralizing RAGE antibody in obese Type 2 diabetic mice. *Diabetes* 53: 166–172, 2004.
29. Fontayne A, Dang PM, Gougerot–Pocidalo MA, and El–Benna J. Phosphorylation of p47phox sites by PKC alpha, beta II, delta, and zeta: effect on binding to p22phox and on NADPH oxidase activation. *Biochemistry* 41: 7743–7750, 2002.
30. Forbes JM, Cooper ME, Thallas V, Burns WC, Thomas MC, Brammar GC, Lee F, Grant SL, Burrell LA, Jerums G, and Osicka TM. Reduction of the accumulation of advanced glycation end products by ACE inhibition in experimental diabetic nephropathy. *Diabetes* 51: 3274–3282, 2002.
31. Forbes JM, Coughlan MT, Fukami K, Laskowski A, Thallas–Bonke V, Dinh D, Long DM, Brownlee M, Cooper ME, and Thorburn DR. Do extracellular (circulating or deitary) advanced glycation end products mediate mitochondrial dysfunction in the kidney? *Diabetologia* 48 Suppl. 1 (Abstract): A91, 2005.
32. Forbes JM, Soulis T, Thallas V, Panagiotopoulos S, Long DM, Vasan S, Wagle D, Jerums G, and Cooper ME. Renoprotective effects of a novel inhibitor of advanced glycation. *Diabetologia* 44: 108–114, 2001.
33. Fujii J, Myint T, Okado A, Kaneto H, and Taniguchi N. Oxidative stress caused by glycation of Cu,Zn-superoxide dismutase and its effects on intracellular components. *Nephrol Dial Transplant* 11 Suppl 5: 34–40, 1996.
34. Gabbay KH, Merola LO, and Field RA. Sorbitol pathway: presence in nerve and cord with substrate accumulation in diabetes. *Science* 151: 209–210, 1966.
35. Gambara V, Mecca G, Remuzzi G, and Bertani T. Heterogeneous nature of renal lesions in Type II diabetes. *J Am Soc Nephrol* 3: 1458–1466, 1993.
36. Gorin Y, Block K, Hernandez J, Bhandari B, Wagner B, Barnes JL, and Abboud HE. Nox4 NAD(P)H oxidase mediates hypertrophy and fibronectin expression in the diabetic kidney. *J Biol Chem* 280: 39616–39626, 2005.
37. Greene DA, Arezzo JC, and Brown MB. Effect of aldose reductase inhibition on nerve conduction and morphometry in diabetic neuropathy. Zenarestat Study Group. *Neurology* 53: 580–591, 1999.
38. Griendling KK, Sorescu D, and Ushio–Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res* 86: 494–501, 2000.
39. Griendling KK and Ushio–Fukai M. Reactive oxygen species as mediators of angiotensin II signaling. *Regul Pept* 91: 21–27, 2000.
40. Guzik TJ, West NE, Black E, McDonald D, Ratnatunga C, Pillai R, and Channon KM. Vascular superoxide production by NAD(P)H oxidase: association with endothelial dysfunction and clinical risk factors. *Circ Res* 86: E85–90, 2000.
41. Ha H, Yu MR, Choi YJ, Kitamura M, and Lee HB. Role of high glucose-induced nuclear factor-kappaB activation in monocyte chemoattractant protein-1 expression by mesangial cells. *J Am Soc Nephrol* 13: 894–902, 2002.
42. Haitoglou CS, Tsilibary EC, Brownlee M, and Charonis AS. Altered cellular interactions between endothelial cells and nonenzymatically glucosylated laminin/Type IV collagen. *J Biol Chem* 267: 12404–12407, 1992.
43. Hamada Y, Araki N, Koh N, Nakamura J, Horiuchi S, and Hotta N. Rapid formation of advanced glycation end products by intermediate metabolites of glycolytic pathway and polyol pathway. *Biochem Biophys Res Commun* 228: 539–543, 1996.
44. Heales SJ and Bolanos JP. Impairment of brain mitochondrial function by reactive nitrogen species: the role of glutathione in dictating susceptibility. *Neurochem Int* 40: 469–474, 2002.
45. Heilig CW, Concepcion LA, Riser BL, Freytag SO, Zhu M, and Cortes P. Overexpression of glucose transporters in rat mesangial cells cultured in a normal glucose milieu mimics the diabetic phenotype. *J Clin Invest* 96: 1802–1814, 1995.
46. Hori O, Yan SD, Ogawa S, Kuwabara K, Matsumoto M, Stern D, and Schmidt AM. The receptor for advanced glycation end-products has a central role in mediating the effects of advanced glycation end-products on the development of vascular disease in diabetes mellitus. *Nephrol Dial Transplant* 11 Suppl 5: 13–16, 1996.
47. Horie K, Miyata T, Maeda K, Miyata S, Sugiyama S, Sakai H, van Ypersole de Strihou C, Monnier VM, Witztum JL, and Kurokawa K. 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* 100: 2995–3004, 1997.
48. Hotta N, Toyota T, Matsuoka K, Shigeta Y, Kikkawa R, Kaneko T, Takahashi A, Sugimura K, Koike Y, Ishii J, and Sakamoto N. Clinical efficacy of fidarestat, a novel aldose reductase inhibitor, for diabetic peripheral neuropathy: a 52-week multicenter placebo-controlled double-blind parallel group study. *Diabetes Care* 24: 1776–1782, 2001.
49. Hovind P, Tarnow L, Rossing K, Rossing P, Eising S, Larsen N, Binder C, and Parving HH. Decreasing incidence of severe diabetic microangiopathy in Type 1 diabetes. *Diabetes Care* 26: 1258–1264, 2003.
50. Hua H, Munk S, Goldberg H, Fantus IG, and Whiteside CI. High glucose-suppressed endothelin-1 Ca2+ signaling via NADPH oxidase and diacylglycerol-sensitive protein kinase C isozymes in mesangial cells. *J Biol Chem* 278: 33951–33962, 2003.
51. Huang JS, Guh JY, Chen HC, Hung WC, Lai YH, and Chuang LY. Role of receptor for advanced glycation end-product (RAGE) and the JAK/STAT-signaling pathway in AGE-induced collagen production in NRK-49F cells. *J Cell Biochem* 81: 102–113, 2001.
52. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H, and 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* 49: 1939–1945, 2000.
53. Inoguchi T, Sonta T, Tsubouchi H, Etoh T, Kakimoto M, Sonoda N, Sato N, Sekiguchi N, Kobayashi K, Sumimoto H, Utsumi H, and 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* 14: S227–232, 2003.
54. Ishii H, Jirousek MR, Koya D, Takagi C, Xia P, Clermont A, Bursell SE, Kern TS, Ballas LM, Heath WF, Stramm LE, Feener EP, and King GL. Amelioration of vascular dysfunctions in diabetic rats by an oral PKC beta inhibitor. *Science* 272: 728–731, 1996.
55. James AM and Murphy MP. How mitochondrial damage affects cell function. *J Biomed Sci* 9: 475–487, 2002.
56. Jerums G, Panagiotopoulos S, Forbes J, Osicka T, and Cooper M. Evolving concepts in advanced glycation, diabetic nephropathy, and diabetic vascular disease. *Arch Biochem Biophys* 419: 55–62, 2003.

57. Johansen JS, Harris AK, Rychly DJ, and Ergul A. Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. *Cardiovasc Diabetol* 4: 5, 2005.
58. Jones SA, Hancock JT, Jones OT, Neubauer A, and Topley N. The expression of NADPH oxidase components in human glomerular mesangial cells: detection of protein and mRNA for p47phox, p67phox, and p22phox. *J Am Soc Nephrol* 5: 1483–1491, 1995.
59. Kador PF. The role of aldose reductase in the development of diabetic complications. *Med Res Rev* 8: 325–352, 1988.
60. Kaiser N, Sasson S, Feener EP, Boukobza–Vardi N, Higashi S, Moller DE, Davidheiser S, Przybylski RJ, and King GL. Differential regulation of glucose transport and transporters by glucose in vascular endothelial and smooth muscle cells. *Diabetes* 42: 80–89, 1993.
61. Kedziora–Kornatowska K, Szram S, Kornatowski T, Szadujkis–Szadurski L, Kedziora J, and Bartosz G. Effect of vitamin E and vitamin C supplementation on antioxidative state and renal glomerular basement membrane thickness in diabetic kidney. *Nephron Exp Nephrol* 95: e134–143, 2003.
62. Kelly DJ, Gilbert RE, Cox AJ, Soulis T, Jerums G, and Cooper ME. Aminoguanidine ameliorates overexpression of prosclerotic growth factors and collagen deposition in experimental diabetic nephropathy. *J Am Soc Nephrol* 12: 2098–2107, 2001.
63. Kitada M, Koya D, Sugimoto T, Isono M, Araki S, Kashiwagi A, and Haneda M. Translocation of glomerular p47phox and p67phox by protein kinase C-beta activation is required for oxidative stress in diabetic nephropathy. *Diabetes* 52: 2603–2614, 2003.
64. Klahr S. Progression of chronic renal disease. *Heart Dis* 3: 205–209, 2001.
65. Koopman WJ, Verkaart S, Visch HJ, van der Westhuizen FH, Murphy MP, van den Heuvel LW, Smeitink JA, and Willems PH. Inhibition of complex I of the electron transport chain causes O2-. -mediated mitochondrial outgrowth. *Am J Physiol Cell Physiol* 288: C1440–1450, 2005.
66. Koya D, Haneda M, Nakagawa H, Isshiki K, Sato H, Maeda S, Sugimoto T, Yasuda H, Kashiwagi A, Ways DK, King GL, and Kikkawa R. Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC beta inhibitor in diabetic db/db mice, a rodent model for Type 2 diabetes. *FASEB J* 14: 439–447, 2000.
67. Koya D, Jirousek MR, Lin YW, Ishii H, Kuboki K, and King GL. Characterization of protein kinase C beta isoform activation on the gene expression of transforming growth factor-beta, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats. *J Clin Invest* 100: 115–126, 1997.
68. Koya D and King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes* 47: 859–866, 1998.
69. Kubo E, Urakami T, Fatma N, Akagi Y, and Singh DP. Polyol pathway-dependent osmotic and oxidative stresses in aldose reductase-mediated apoptosis in human lens epithelial cells: role of AOP2. *Biochem Biophys Res Commun* 314: 1050–1056, 2004.
70. Lee AY and Chung SS. Contributions of polyol pathway to oxidative stress in diabetic cataract. *FASEB J* 13: 23–30, 1999.
71. Li J and Schmidt AM. Characterization and functional analysis of the promoter of RAGE, the receptor for advanced glycation end products. *J Biol Chem* 272: 16498–16506, 1997.
72. Li JM and Shah AM. ROS generation by nonphagocytic NADPH oxidase: potential relevance in diabetic nephropathy. *J Am Soc Nephrol* 14: S221–226, 2003.
73. Lin CL, Wang FS, Kuo YR, Huang YT, Huang HC, Sun YC, and Kuo YH. Ras modulation of superoxide activates ERK-dependent fibronectin expression in diabetes-induced renal injuries. *Kidney Int* 69: 1593–1600, 2006.
74. Malherbe P, Richards JG, Gaillard H, Thompson A, Diener C, Schuler A, and Huber G. cDNA cloning of a novel secreted isoform of the human receptor for advanced glycation end products and characterization of cells co-expressing cell-surface scavenger receptors and Swedish mutant amyloid precursor protein. *Brain Res Mol Brain Res* 71: 159–170, 1999.
75. McRobert EA, Gallicchio M, Jerums G, Cooper ME, and Bach LA. The amino-terminal domains of the ezrin, radixin, and moesin (ERM) proteins bind advanced glycation end products, an interaction that may play a role in the development of diabetic complications. *J Biol Chem* 278: 25783–25789, 2003.
76. Misur I, Zarkovic K, Barada A, Batelja L, Milicevic Z, and Turk Z. Advanced glycation endproducts in peripheral nerve in Type 2 diabetes with neuropathy. *Acta Diabetol* 41: 158–166, 2004.
77. Miura J, Yamagishi S, Uchigata Y, Takeuchi M, Yamamoto H, Makita Z, and Iwamoto Y. Serum levels of non-carboxymethyllysine advanced glycation endproducts are correlated to severity of microvascular complications in patients with Type 1 diabetes. *J Diabetes Complications* 17: 16–21, 2003.
78. Miyata T, Hori O, Zhang J, Yan SD, Ferran L, Iida Y, and Schmidt AM. The receptor for advanced glycation end products (RAGE) is a central mediator of the interaction of AGE-beta2microglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway. Implications for the pathogenesis of dialysis-related amyloidosis. *J Clin Invest* 98: 1088–1094, 1996.
79. Miyata T, Inagi R, Iida Y, Sato M, Yamada N, Oda O, Maeda K, and Seo H. Involvement of beta 2-microglobulin modified with advanced glycation end products in the pathogenesis of hemodialysis-associated amyloidosis. Induction of human monocyte chemotaxis and macrophage secretion of tumor necrosis factor-alpha and interleukin-1. *J Clin Invest* 93: 521–528, 1994.
80. Miyata T, van Ypersele de Strihou C, Ueda Y, Ichimori K, Inagi R, Onogi H, Ishikawa N, Nangaku M, and Kurokawa K. Angiotensin II receptor antagonists and angiotensin-converting enzyme inhibitors lower *in vitro* the formation of advanced glycation end products: biochemical mechanisms. *J Am Soc Nephrol* 13: 2478–2487, 2002.
81. Monnier VM, Bautista O, Kenny D, Sell DR, Fogarty J, Dahms W, Cleary PA, Lachin J, and Genuth S. Skin collagen glycation, glycoxidation, and crosslinking are lower in subjects with long-term intensive versus conventional therapy of Type 1 diabetes: relevance of glycated collagen products versus HbA1c as markers of diabetic complications. DCCT Skin Collagen Ancillary Study Group. Diabetes Control and Complications Trial. *Diabetes* 48: 870–880, 1999.
82. Monnier VM, Sell DR, Nagaraj RH, Miyata S, Grandhee S, Odetti P, and Ibrahim SA. Maillard reaction-mediated molecular damage to extracellular matrix and other tissue proteins in diabetes, aging, and uremia. *Diabetes* 41 Suppl 2: 36–41, 1992.
83. Mott JD, Khalifah RG, Nagase H, Shield CF, 3rd, Hudson JK, and Hudson BG. Nonenzymatic glycation of Type IV collagen and matrix metalloproteinase susceptibility. *Kidney Int* 52: 1302–1312, 1997.
84. Naka Y, Bucciarelli LG, Wendt T, Lee LK, Rong LL, Ramasamy R, Yan SF, and Schmidt AM. RAGE axis: Animal models and novel insights into the vascular complications of diabetes. *Arterioscler Thromb Vasc Biol* 24: 1342–1349, 2004.
85. Narayan KM, Boyle JP, Thompson TJ, Sorensen SW, and Williamson DF. Lifetime risk for diabetes mellitus in the United States. *JAMA* 290: 1884–1890, 2003.
86. Nathan DM, Lachin J, Cleary P, Orchard T, Brillon DJ, Backlund JY, O'Leary DH, and Genuth S. Intensive diabetes therapy and carotid intima-media thickness in Type 1 diabetes mellitus. *N Engl J Med* 348: 2294–2303, 2003.
87. Neeper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YC, Elliston K, Stern D, and Shaw A. Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem* 267: 14998–15004, 1992.
88. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, and Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404: 787–790, 2000.
89. Obrosova IG. Increased sorbitol pathway activity generates oxidative stress in tissue sites for diabetic complications. *Antioxid Redox Signal* 7: 1543–1552, 2005.
90. Onozato ML, Tojo A, Goto A, Fujita T, and Wilcox CS. Oxidative stress and nitric oxide synthase in rat diabetic nephropathy: effects of ACEI and ARB. *Kidney Int* 61: 186–194, 2002.
91. Ortwerth BJ and James HL. Lens proteins block the copper-mediated formation of reactive oxygen species during glycation reactions *in vitro. Biochem Biophys Res Commun* 259: 706–710, 1999.

92. Osicka TM, Houlihan CA, Chan JG, Jerums G, and Comper WD. Albuminuria in patients with Type 1 diabetes is directly linked to changes in the lysosome-mediated degradation of albumin during renal passage. *Diabetes* 49: 1579–1584, 2000.
93. Park L, Raman KG, Lee KJ, Lu Y, Ferran LJ Jr, Chow WS, Stern D, and Schmidt AM. Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. *Nat Med* 4: 1025–1031, 1998.
94. Pitkanen S and Robinson BH. Mitochondrial complex I deficiency leads to increased production of superoxide radicals and induction of superoxide dismutase. *J Clin Invest* 98: 345–351, 1996.
95. Radeke HH, Cross AR, Hancock JT, Jones OT, Nakamura M, Kaever V and Resch K. Functional expression of NADPH oxidase components (alpha- and beta-subunits of cytochrome b558 and 45-kDa flavoprotein) by intrinsic human glomerular mesangial cells. *J Biol Chem* 266: 21025–21029, 1991.
96. Ritz E, Keller C, Bergis K, and Strojek K. Pathogenesis and course of renal disease in IDDM/NIDDM: differences and similarities. *Am J Hypertens* 10: 202S–207S, 1997.
97. Rosca MG, Mustata TG, Kinter MT, Ozdemir AM, Kern TS, Szweda LI, Brownlee M, Monnier VM, and Weiss MF. Glycation of mitochondrial proteins from diabetic rat kidney is associated with excess superoxide formation. *Am J Physiol Renal Physiol* 289: F420–430, 2005.
98. Rosen P, Nawroth PP, King G, Moller W, Tritschler HJ, and Packer L. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. *Diabetes Metab Res Rev* 17: 189–212, 2001.
99. Rotig A, de Lonlay P, Chretien D, Foury F, Koenig M, Sidi D, Munnich A, and Rustin P. Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia. *Nat Genet* 17: 215–217, 1997.
100. Ruggenenti P and Remuzzi G. Nephropathy of Type 1 and Type 2 diabetes: diverse pathophysiology, same treatment? *Nephrol Dial Transplant* 15: 1900–1902, 2000.
101. Saito A, Nagai R, Tanuma A, Hama H, Cho K, Takeda T, Yoshida Y, Toda T, Shimizu F, Horiuchi S, and Gejyo F. Role of megalin in endocytosis of advanced glycation end products: implications for a novel protein binding to both megalin and advanced glycation end products. *J Am Soc Nephrol* 14: 1123–1131, 2003.
102. Schmidt AM, Hori O, Chen JX, Li JF, Crandall J, Zhang J, Cao R, Yan SD, Brett J, and Stern D. Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. A potential mechanism for the accelerated vasculopathy of diabetes. *J Clin Invest* 96: 1395–1403, 1995.
103. Schmidt AM, Vianna M, Gerlach M, Brett J, Ryan J, Kao J, Esposito C, Hegarty H, Hurley W, Clauss M, and et al. Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface. *J Biol Chem* 267: 14987–14997, 1992.
104. Schmidt AM, Yan SD, Wautier JL, and Stern D. Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circ Res* 84: 489–497, 1999.
105. Sell DR, Lapolla A, Odetti P, Fogarty J, and Monnier VM. Pentosidine formation in skin correlates with severity of complications in individuals with long-standing IDDM. *Diabetes* 41: 1286–1292, 1992.
106. Shinohara M, Thornalley PJ, Giardino I, Beisswenger P, Thorpe SR, Onorato J, and Brownlee M. Overexpression of glyoxalase-I in bovine endothelial cells inhibits intracellular advanced glycation endproduct formation and prevents hyperglycemia-induced increases in macromolecular endocytosis. *J Clin Invest* 101: 1142–1147, 1998.
107. Shiose A, Kuroda J, Tsuruya K, Hirai M, Hirakata H, Naito S, Hattori M, Sakaki Y, and Sumimoto H. A novel superoxide-producing NAD(P)H oxidase in kidney. *J Biol Chem* 276: 1417–1423, 2001.
108. Silbiger S, Crowley S, Shan Z, Brownlee M, Satriano J, and Schlondorff D. Nonenzymatic glycation of mesangial matrix and prolonged exposure of mesangial matrix to elevated glucose reduces collagen synthesis and proteoglycan charge. *Kidney Int* 43: 853–864, 1993.
109. Smedsrod B, Melkko J, Araki N, Sano H, and Horiuchi S. Advanced glycation end products are eliminated by scavenger-receptor-mediated endocytosis in hepatic sinusoidal Kupffer and endothelial cells. *Biochem J* 322: 567–573, 1997.
110. Soulis T, Cooper ME, Sastra S, Thallas V, Panagiotopoulos S, Bjerrum OJ, and Jerums G. Relative contributions of advanced glycation and nitric oxide synthase inhibition to aminoguanidine-mediated renoprotection in diabetic rats. *Diabetologia* 40: 1141–1151, 1997.
111. Soulis–Liparota T, Cooper M, Papazoglou D, Clarke B, and Jerums G. Retardation by aminoguanidine of development of albuminuria, mesangial expansion, and tissue fluorescence in streptozocin-induced diabetic rat. *Diabetes* 40: 1328–1334, 1991.
112. Stitt AW, Li YM, Gardiner TA, Bucala R, Archer DB, and Vlassara H. Advanced glycation end products (AGEs) co-localize with AGE receptors in the retinal vasculature of diabetic and of AGE-infused rats. *Am J Pathol* 150: 523–531, 1997.
113. Suarez G, Rajaram R, Oronsky AL, and Gawinowicz MA. Nonenzymatic glycation of bovine serum albumin by fructose (fructation). Comparison with the Maillard reaction initiated by glucose. *J Biol Chem* 264: 3674–3679, 1989.
114. Sugiyama S, Miyata T, Ueda Y, Tanaka H, Maeda K, Kawashima S, Van Ypersele de Strihou C, and Kurokawa K. Plasma levels of pentosidine in diabetic patients: an advanced glycation end product. *J Am Soc Nephrol* 9: 1681–1688, 1998.
115. Susztak K, Raff AC, Schiffer M, and Bottinger EP. Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. *Diabetes* 55: 225–233, 2006.
116. Tan KC, Chow WS, Tam S, Bucala R, and Betteridge J. Association between acute-phase reactants and advanced glycation end products in Type 2 diabetes. *Diabetes Care* 27: 223–228, 2004.
117. Tanji N, Markowitz GS, Fu C, Kislinger T, Taguchi A, Pischetsrieder M, Stern D, Schmidt AM, and D'Agati VD. Expression of advanced glycation end products and their cellular receptor RAGE in diabetic nephropathy and nondiabetic renal disease. *J Am Soc Nephrol* 11: 1656–1666, 2000.
118. Thallas–Bonke V, Lindschau C, Rizkalla B, Bach LA, Boner G, Meier M, Haller H, Cooper ME, and Forbes JM. Attenuation of extracellular matrix accumulation in diabetic nephropathy by the advanced glycation end product cross-link breaker ALT-711 via a protein kinase C-alpha-dependent pathway. *Diabetes* 53: 2921–2930, 2004.
119. Thornalley PJ. The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. *Biochem J* 269: 1–11, 1990.
120. Thornalley PJ, Langborg A, and Minhas HS. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem J* 344: 109–116, 1999.
121. Twigg SM, Chen MM, Joly AH, Chakrapani SD, Tsubaki J, Kim HS, Oh Y, and Rosenfeld RG. Advanced glycosylation end products up-regulate connective tissue growth factor (insulin-like growth factor-binding protein-related protein 2) in human fibroblasts: a potential mechanism for expansion of extracellular matrix in diabetes mellitus. *Endocrinology* 142: 1760–1769, 2001.
122. Vlassara H. The AGE-receptor in the pathogenesis of diabetic complications. *Diabetes Metab Res Rev* 17: 436–443, 2001.
123. Vlassara H. Receptor-mediated interactions of advanced glycosylation end products with cellular components within diabetic tissues. *Diabetes* 41 Suppl 2: 52–56, 1992.
124. Wautier JL, Wautier MP, Schmidt AM, Anderson GM, Hori O, Zoukourian C, Capron L, Chappey O, Yan SD, Brett J, and et al. Advanced glycation end products (AGEs) on the surface of diabetic erythrocytes bind to the vessel wall via a specific receptor inducing oxidant stress in the vasculature: a link between surface-associated AGEs and diabetic complications. *Proc Natl Acad Sci USA* 91: 7742–7746, 1994.

125. Wautier JL, Zoukourian C, Chappey O, Wautier MP, Guillausseau PJ, Cao R, Hori O, Stern D, and Schmidt AM. Receptor-mediated endothelial cell dysfunction in diabetic vasculopathy. Soluble receptor for advanced glycation end products blocks hyperpermeability in diabetic rats. *J Clin Invest* 97: 238–243, 1996.
126. Wautier MP, Chappey O, Corda S, Stern DM, Schmidt AM, and Wautier JL. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. *Am J Physiol Endocrinol Metab* 280: E685–694, 2001.
127. Wells-Knecht KJ, Zyzak DV, Litchfield JE, Thorpe SR, and Baynes JW. Mechanism of autoxidative glycosylation: identification of glyoxal and arabinose as intermediates in the autoxidative modification of proteins by glucose. *Biochemistry* 34: 3702–3709, 1995.
128. Wendt T, Bucciarelli L, Qu W, Lu Y, Yan SF, Stern DM, and Schmidt AM. Receptor for advanced glycation endproducts (RAGE) and vascular inflammation: insights into the pathogenesis of macrovascular complications in diabetes. *Curr Atheroscler Rep* 4: 228–237, 2002.
129. Wendt T, Tanji N, Guo J, Hudson BI, Bierhaus A, Ramasamy R, Arnold B, Nawroth PP, Yan SF, D'Agati V, and Schmidt AM. Glucose, glycation, and RAGE: implications for amplification of cellular dysfunction in diabetic nephropathy. *J Am Soc Nephrol* 14: 1383–1395, 2003.
130. Wendt TM, Tanji N, Guo J, Kislinger TR, Qu W, Lu Y, Bucciarelli LG, Rong LL, Moser B, Markowitz GS, Stein G, Bierhaus A, Liliensiek B, Arnold B, Nawroth PP, Stern DM, D'Agati VD, and Schmidt AM. RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy. *Am J Pathol* 162: 1123–1137, 2003.
131. Xia P, Inoguchi T, Kern TS, Engerman RL, Oates PJ, and King GL. Characterization of the mechanism for the chronic activation of diacylglycerol-protein kinase C pathway in diabetes and hypergalactosemia. *Diabetes* 43: 1122–1129, 1994.
132. Yagihashi S. Pathogenetic mechanisms of diabetic neuropathy: lessons from animal models. *J Peripher Nerv Syst* 2: 113–132, 1997.
133. Yamamoto Y, Kato I, Doi T, Yonekura H, Ohashi S, Takeuchi M, Watanabe T, Yamagishi S, Sakurai S, Takasawa S, Okamoto H, and Yamamoto H. Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice. *J Clin Invest* 108: 261–268, 2001.
134. Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, and Stern D. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J Biol Chem* 269: 9889–9897, 1994.
135. Yang CW, Vlassara H, Peten EP, He CJ, Striker GE, and Striker LJ. Advanced glycation end products up-regulate gene expression found in diabetic glomerular disease. *Proc Natl Acad Sci USA* 91: 9436–9440, 1994.
136. Yang S, Madyastha P, Bingel S, Ries W, and Key L. A new superoxide-generating oxidase in murine osteoclasts. *J Biol Chem* 276: 5452–5458, 2001.
137. Yokoyama H, Okudaira M, Otani T, Sato A, Miura J, Takaike H, Yamada H, Muto K, Uchigata Y, Ohashi Y, and Iwamoto Y. Higher incidence of diabetic nephropathy in Type 2 than in Type 1 diabetes in early-onset diabetes in Japan. *Kidney Int* 58: 302–311, 2000.
138. Yonekura H, Yamamoto Y, Sakurai S, Petrova RG, Abedin MJ, Li H, Yasui K, Takeuchi M, Makita Z, Takasawa S, Okamoto H, Watanabe T, and Yamamoto H. Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. *Biochem J* 370: 1097–1109, 2003.
139. Zalba G, San Jose G, Moreno MU, Fortuno MA, Fortuno A, Beaumont FJ, and Diez J. Oxidative stress in arterial hypertension: role of NAD(P)H oxidase. *Hypertension* 38: 1395–1399, 2001.
140. Zimmet P, Alberti KG, and Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 414: 782–787, 2001.

Address reprint requests to:
Dr. Melinda T. Coughlan
Albert Einstein Centre for Diabetes Complications
Baker Heart Research Institute
P.O. Box 6492
St. Kilda Road Central
Melbourne 8008, Australia

E-mail: melinda.coughlan@baker.edu.au

Date of first submission to ARS Central, September 22, 2006; date of acceptance, September 26, 2006.

This article has been cited by:

1. Shuhong Qian, Dongxia Huo, Shijin Wang, Qingwen Qian. 2011. Inhibition of glucose-induced vascular endothelial growth factor expression by Salvia miltiorrhiza hydrophilic extract in human microvascular endothelial cells: Evidence for mitochondrial oxidative stress. *Journal of Ethnopharmacology* . [CrossRef]
2. Jeffrey L Barnes, Yves Gorin. 2011. Myofibroblast differentiation during fibrosis: role of NAD(P)H oxidases. *Kidney International* **79**:9, 944-956. [CrossRef]
3. Daolin Tang , Rui Kang , Herbert J. Zeh III , Michael T. Lotze . 2011. High-Mobility Group Box 1, Oxidative Stress, and Disease. *Antioxidants & Redox Signaling* **14**:7, 1315-1335. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. John J. Mieyal , Molly M. Gallogly , Suparna Qanungo , Elizabeth A. Sabens , Melissa D. Shelton . 2008. Molecular Mechanisms and Clinical Implications of Reversible Protein S-Glutathionylation. *Antioxidants & Redox Signaling* **10**:11, 1941-1988. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
5. D GELAIN, M PASQUALI, F CAREGNATO, A ZANOTTOFILHO, J MOREIRA. 2008. Retinol up-regulates the receptor for advanced glycation endproducts (RAGE) by increasing intracellular reactive species. *Toxicology in Vitro* **22**:5, 1123-1127. [CrossRef]
6. José Augusto Nogueira-Machado, Miriam Martins Chaves. 2008. From hyperglycemia to AGE-RAGE interaction on the cell surface: A dangerous metabolic route for diabetic patients. *Expert Opinion on Therapeutic Targets* **12**:7, 871-882. [CrossRef]
7. Emiko Manabe, Osamu Handa, Yuji Naito, Katsura Mizushima, Satomi Akagiri, Satoko Adachi, Tomohisa Takagi, Satoshi Kokura, Takashi Maoka, Toshikazu Yoshikawa. 2008. Astaxanthin protects mesangial cells from hyperglycemia-induced oxidative signaling. *Journal of Cellular Biochemistry* **103**:6, 1925-1937. [CrossRef]
8. Weihai Ying . 2008. NAD+/NADH and NADP+/NADPH in Cellular Functions and Cell Death: Regulation and Biological Consequences. *Antioxidants & Redox Signaling* **10**:2, 179-206. [Citation] [Full Text PDF] [Full Text PDF with Links]
9. Dr. Eiichi Araki , Jun-Ichi Miyazaki . 2007. Metabolic Disorders in Diabetes Mellitus: Impact of Mitochondrial Function and Oxidative Stress on Diabetes and Its Complications. *Antioxidants & Redox Signaling* **9**:3, 289-291. [Citation] [Full Text PDF] [Full Text PDF with Links]