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Forum Review

Mechanisms for Oxidative Stress in Diabetic Cardiovascular Disease

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ABSTRACT

Obesity, metabolic syndrome, and diabetes are increasingly prevalent in Western society, and they markedly increase the risk for atherosclerotic vascular disease, the major cause of death in diabetics. Although recent evidence suggests a causal role for oxidative stress in insulin resistance, diabetes, and atherosclerosis, there is considerable controversy regarding its nature, magnitude, and underlying mechanisms. Glucose promotes glycoxidation reactions *in vitro,* and products of glycoxidation and lipoxidation are elevated in plasma and tissue from humans suffering from diabetes, but the exact relationships between hyperglycemia and oxidative stress are poorly understood. This review focuses on molecular mechanisms of increased oxidative stress in diabetes, the relationship of oxidant production to hyperglycemia, and the potential interaction of reactive carbonyls and lipids in oxidant generation. Using highly sensitive and specific gas chromatography–mass spectrometry, molecular signatures of specific oxidation pathways were identified in tissues of diabetic humans and animals. These studies support the hypothesis that unique reactive intermediates generated in localized microenvironments of vulnerable tissues promote diabetic damage. Therapies interrupting these reactive pathways in vascular tissue might help prevent cardiovascular disease in this high-risk population. *Antioxid. Redox Signal.* 9, 955–969.

INTRODUCTION

DIABETES MELLITUS is a serious global health problem. A recent study by the World Health Organization estimated that the worldwide prevalence of this disease is expected to grow from 171 million in 2000 to 366 million by 2030 (142). Roughly 21 million people in the United States (7%) are afflicted by this disorder, and 54 million are at risk. Moreover, type 2 diabetes is being diagnosed more frequently in children and adolescents, particularly in American Indians, African Americans, and Hispanic/Latino Americans. Twenty percent of Americans over the age of 60 are diabetic, making the disease one of the most prevalent disorders in the geriatric population (3).

Hyperglycemia is the metabolic hallmark of both type 1 and type 2 diabetes. Type 1, which accounts for 5–10% of all cases in the United States, develops when the immune system destroys the pancreatic β-cells, drastically decreasing insulin production. Type 2, which accounts for 90% of cases, is a complex, multisystem disease characterized by both insulin resistance in insulin-sensitive tissues (skeletal muscle, liver, and adipose tissue) and progressive pancreatic β-cell dysfunction. These deficits ultimately result in too little insulin to maintain blood glucose levels in the normal range.

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 diabetes. Diabetic retinopathy is the major cause of adult blindness in the United States. Diabetic neuropathy, which affects roughly half of all diabetic patients is the most common cause of nontraumatic amputations (38) and diabetic nephropathy is the major cause of end-stage renal disease (24).

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Macrovascular complications due to atherosclerosis remain the leading cause of death in diabetic humans. Myocardial infarction, stroke, and peripheral vascular disease are two to four times more prevalent in such patients (24). Moreover, atherosclerosis occurs earlier, and follows a more aggressive course (45). Thus, the cardiovascular event rate in diabetic patients without documented coronary artery disease (CAD) is equivalent to that of nondiabetic patients with CAD (45, 46). Moreover, diabetic patients have higher mortality following myocardial infarction than nondiabetic subjects (45). Women with diabetes lose their premenopausal cardioprotection, and are vulnerable to CAD at the same rate as men (62).

Whereas tissue-specific factors may accentuate diabetic damage, it has become increasingly clear that microvascular and macrovascular diseases share a common pathophysiology. During the past two decades, considerable evidence has implicated oxidative stress in several distinct conditions, including aging, atherosclerosis, neurodegenerative diseases, diabetes, and end-stage renal disease (reviewed in Refs. 4, 5, 15, 56, 72, 123) Oxidative stress occurs when there is an imbalance in the relative rates of oxidant generation and oxidant scavenging, with a subsequent increase in the level of oxidized biomolecules and associated tissue damage.

Accumulating evidence points to hyperglycemia-induced increases in the production of oxidants and other reactive intermediates as one important mechanism for the initiation and progression of endothelial dysfunction in diabetic subjects (16, 61). Recent advances in our understanding of oxidative stress, insulin resistance, and diabetes have focused on mass spectrometric identification of oxidative modifications to tissue proteins and lipids, characterization of redox signaling pathways that are activated by oxidative stress and diabetes, the central role for oxidative stress in all forms of insulin resistance (61), identification of specific cellular organelles (such as mitochondria) and enzymes (such as myeloperoxidase) that generate reactive intermediates, and the discovery that oxidants can activate apoptotic pathways.

In this review, we discuss the proposal that oxidative stress might play a causal role in both atherosclerosis and diabetes, and consider how glucose-induced oxidative stress may lead to microvascular and cardiovascular complications. We will also cover the potential relationship of hyperglycemia and lipids to oxidant-generating pathways and the rationale for therapies aimed at decreasing oxidative stress. One key issue has been to identify specific oxidative pathways that contribute to diabetic complications. Our overall strategy has been to develop sensitive and specific mass spectrometric methods for defining molecular fingerprints of oxidative pathways *in vitro* and, using those tools, to collect evidence that those pathways are pathophysiologically relevant *in vivo*. This is a critical prelude to rationally designing and testing antioxidant therapies.

PATHOPHYSIOLOGY OF ATHEROSCLEROSIS AND DIABETIC COMPLICATIONS

Inflammation, oxidative stress, and atherosclerosis

Atherosclerosis is a chronic inflammatory disease characterized by infiltration of lipids and inflammatory cells, such as monocyte-derived macrophages and T-lymphocytes, into the artery wall (117). Although it is well known that elevated levels of low-density lipoprotein (LDL) greatly increase the risk for atherosclerosis (14), *in vitro* studies suggest that LDL by itself is not atherogenic but needs to be modified to initiate atherosclerotic disease (42, 144). This conclusion led to the 'oxidation hypothesis', which proposed that LDL must be oxidatively modified to become atherogenic. Many lines of evidence support this hypothesis. Oxidized LDL is taken up by scavenger receptors of macrophages, which then become lipid-laden foam cells, the pathologic hallmark of early atherosclerotic lesions (129). Oxidized LDL has been isolated from human and animal atherosclerotic tissue, and immunohistochemical studies have detected oxidized lipids in atherosclerotic lesions (44, 146, 147). All major cell types involved in atherosclerosis—smooth muscle cells, endothelial cells, and macrophages—produce reactive oxidants that can oxidize LDL *in vitro* (59, 96, 130). Moreover, oxidized LDL attracts mononuclear cells and stimulates the production of monocyte chemoattractant protein-1 and other inflammatory cytokines, leading to the conversion of fatty streaks to more advanced complex lesions as smooth muscle cells migrate from the media into the subendothelial space. Oxidized LDL may also stimulate smooth cells to synthesize extracellular matrix and activate a signaling cascade by interacting with the lectin-like OxLDL receptor (41, 129). Finally, several structurally unrelated lipid-soluble antioxidants that inhibit LDL oxidation *in vitro* also inhibit atherosclerosis in hypercholesterolemic animals (22, 76, 106).

Hyperglycemia and diabetic complications

Both the degree of glycemic control and the duration of diabetes predict the risk of diabetic complications (79, 98). These observations have given rise to the "glucose hypothesis," which suggests that glucose mediates many of the deleterious effects of the disease. Both the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) found that strict glycemic control dramatically lowered the incidence of retinopathy, nephropathy, and neuropathy (29, 98, 133, 134). The majority of patients involved in the DCCT trial were subsequently followed up in the Epidemiology of Diabetes Interventions and Complications (EDIC) study. Glycemic control in the intensive treatment group and the conventional therapy group were similar during the EDIC follow-up. Interestingly, the DCCT/EDIC study demonstrated that a period of intensive diabetes therapy during the DCCT had a long-term, beneficial effect on the subsequent risk of microvascular complications (30). The pathophysiological mechanisms responsible for the improvement are unclear and are referred to as "metabolic memory."

Diabetes also strongly increases the risk for atherosclerotic macrovascular disease, which is two to four times more common in diabetic men than in the general population and is prevalent even in premenopausal diabetic women. Recent data from the DCCT/EDIC cohort showed that, during the mean 17 years of follow-up, only 46 cardiovascular events occurred in 31 patients who had received intensive treatment in the DCCT, compared with 98 events in 52 patients who had received conventional treatment. Intensive treatment reduced

the risk of any CAD event by 42% and the risk of nonfatal myocardial infarction, stroke, or death from CAD by 57%, strongly suggesting that glycemic control lowers macrovascular disease end points as well (99). Additionally, intensive therapy during the DCCT associated with decreased progression of intima—medial thickness, a surrogate marker for atherosclerosis, 6 years after the end of the trial (100). The UKPDS suggested a trend toward less macrovascular disease with glucose-lowering therapy, but the difference did not reach statistical significance.

These findings suggest that hyperglycemia promotes atherosclerosis and its associated complications. Therefore, glucose itself may be toxic to the artery wall. However, strict glycemic control alone does not prevent diabetic complications, suggesting the involvement of additional factors. Proposed factors include abnormalities in lipoproteins, metabolic derangements (insulin resistance, elevated free fatty acid levels, etc.), and variations in genes controlling lipid metabolism, all of which might be important in macrovascular as well as microvascular disease (122). Moreover, the presence of nephropathy, as measured by microalbuminuria and albuminuria, associated with a significant increase in the risk of CAD in the DCCT/EDIC cohort, suggesting common pathophysiological mechanisms in microvascular and macrovascular disease (99).

How does hyperglycemia lead to diabetic complications?

Several mechanisms have been proposed to explain how hyperglycemia mediates diabetic damage. They include formation of advanced glycosylation end products (AGEs) (5, 6, 15, 16, 20, 120, 131), oxidative stress (5, 6, 95), carbonyl stress (5, 6, 95), increased protein kinase C (PKC) activity (70), altered growth factor or cytokine activities (112, 124), reductive stress or pseudohypoxia (67, 143), and mitochondrial dysfunction (16, 101). Some of these hypotheses overlap. For example, AGEs might promote growth factor expression and oxidative stress, and oxidative stress might promote AGE formation (5, 6, 95). Mitochondrial superoxide can result in oxidative stress, form AGE products, and increase PKC activity (16). It is likely that different tissues are differentially vulnerable to hyperglycemia, depending on which biochemical pathways are affected.

Insulin resistance, visceral adiposity, and CAD

Obesity and visceral adiposity frequently associate with diabetes and insulin resistance. Also, insulin resistance clusters with the metabolic syndrome, a constellation of classic CAD risk factors such as lipid abnormalities, hypertension, and impaired glucose tolerance. Emerging data support the hypothesis that oxidative stress plays a causal role in insulin resistance (61) and might be linked with visceral adiposity. Mature adipocytes function as an endocrine/paracrine organ that secretes numerous adipokines, cytokines, and growth factors, particularly in the setting of insulin resistance. A significant number of these proatherogenic factors can be released from components of visceral fat, such as infiltrating macrophages, that are not adipocytes (139, 140). Several possible mechanisms link obesity to CAD, such as the increased levels of nonesterified fatty acids (NEFA) that result from increased lipolysis and disturbances in adipokine and cytokine secretion. These processes in turn are linked with insulin resistance (135). NEFAs are independently associated with CAD mortality in older men (113). Several adipokines and cytokines, such as adiponectin, interleukin-6 (IL-6), retinol-binding protein-4 (RBP-4), resistin, and tumor necrosis factor-α, are associated with insulin resistance. RBP-4 is an adipocyte-derived molecule that is elevated prior to the onset of diabetes (43), and it appears to impair insulin signaling in muscle and promote insulin resistance (1, 145). Visceral fat releases IL-6, which can contribute to local and systemic inflammation and elevation of C-reactive protein levels (135). Thus, insulin resistance is intricately linked with visceral adiposity and oxidative stress, and it may promote endothelial dysfunction and CAD.

OXIDATIVE STRESS IN DIABETES AND ATHEROSCLEROSIS

Oxidative damage in diabetes and atherosclerosis

Although oxidative stress has a well-established role in diabetic complications and atherosclerosis, its origins, magnitude, and localization are controversial. Moreover, it is not known whether oxidative stress is a primary event that occurs early in the disease or whether it represents a secondary phenomenon that merely reflects end-stage tissue damage (6). This distinction has important clinical relevance. If oxidative stress simply reflects tissue damage, interventions that reduce it may fail to affect the disease process. If oxidative stress promotes tissue injury, therapies that interrupt oxidative pathways early in the disease may prevent complications, and those that act later may slow disease progression.

Mass spectrometry is a powerful approach for detecting biomarkers of oxidative stress in vivo

Immunohistochemistry and dihydroethidium fluorescence have been extensively used to study oxidation-specific epitopes and oxidant production in targets of diabetic damage and atherosclerosis. These techniques are highly sensitive, and their ability to provide structural data can localize oxidative events. However, they are nonspecific and, at best, only semiquantitative. In contrast, mass spectrometry (MS) offers a powerful set of analytical tools for quantifying and identifying biomolecules. Isotope dilution gas chromatography (GC)/MS is a highly sensitive and specific method that we have used to quantify oxidation of specific amino acid markers.

The overall strategy for identifying target analytes by GC/MS is outlined in Fig. 1 (55). Biomolecules such as oxidized amino acids (Fig. 2) derived from plasma or tissue are separated by GC, derivatized and ionized. The mass-to-charge ratios of ions derived by fragmenting the ionized, derivatized parent compound are determined by MS. A full scan mass spectrum obtained for a target analyte, 3-chlorotyrosine (derived by acid hydrolysis from plasma proteins), is shown in Fig. 1. Such a spectrum can unequivocally identify a target biomolecule because each compound has a unique fragmentation pattern. The analyte is quantified by adding a stable,

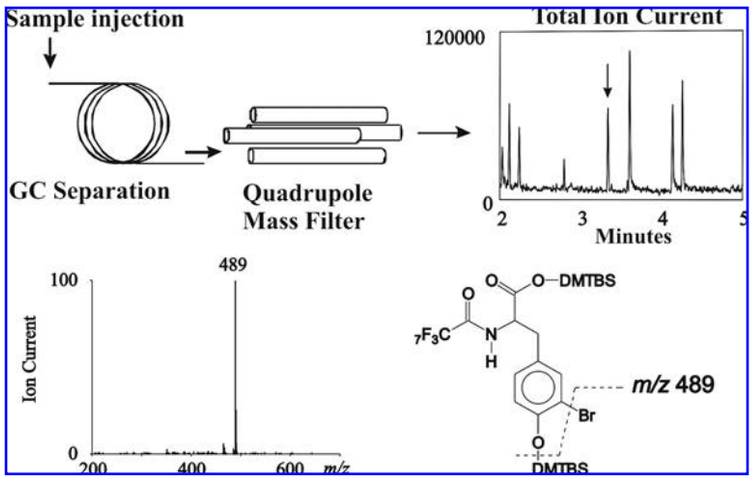

FIG. 1. Schematic representation of gas chromatography–mass spectrometry (GC/MS) for identifying oxidized amino acids. This mass spectrum derived from the dimethylsilyl derivative of 3-chlorotyrosine in protein digests of human plasma is a representative example. Plasma proteins were delipidated and hydrolyzed. Isolated amino acids were derivatized and analyzed by GC/MS.

isotopically labeled internal standard, which is identical to the target analyte except for the heavy isotope. With certain ionization processes, such as electron capture negative-ion chemical ionization, it is possible to detect and quantify subfemtomole levels of biomolecules.

Pathways for generating oxidants

Many pathways oxidize proteins *in vitro*. However, the specific pathways that promote oxidative stress in diabetes have not been conclusively identified. One reason is that oxidizing intermediates are difficult to detect *in vivo* because they are short-lived and generated at low levels. Proposed pathways for increased oxidant generation and oxidative stress in diabetes are outlined in Fig. 3.

The glycoxidation pathway. Collectively, glucose-mediated oxidative reactions are called glycoxidation pathways. In its open-chain form, glucose has a carbonyl group that can be involved in oxidative chemistry. In the presence of oxygen, glucose can auto-oxidize to reactive oxygen species, such as hydroxyl radical, which cross-links proteins (65). Glucose also reacts nonenzymatically with proteins to form the reversible Schiff base adduct, which subsequently can rearrange itself into the stable Amadori product and AGE products.

In vitro, free metal ions catalyze steps in a nonenzymatic glycoxidation pathway that generates AGE products. One important intermediate is hydroxyl radical, which can peroxidize lipids and convert phenylalanine residues of proteins into two unnatural isomers of tyrosine, *ortho*-tyrosine and *meta*-tyrosine (63, 64, 73). Reduced, redox-active metal ions (M^{n+}) such as Fe^{2+} and Cu^{1+} generate hydroxyl radical ($HO^{\bullet}$) when they react with hydrogen peroxide (H_2O_2; Equation 1).

$$M^{n+} + H_2O_2 \rightarrow M^{n+-1} + HO^{\bullet} + HO^{-} \qquad \text{(Eq. 1)}$$

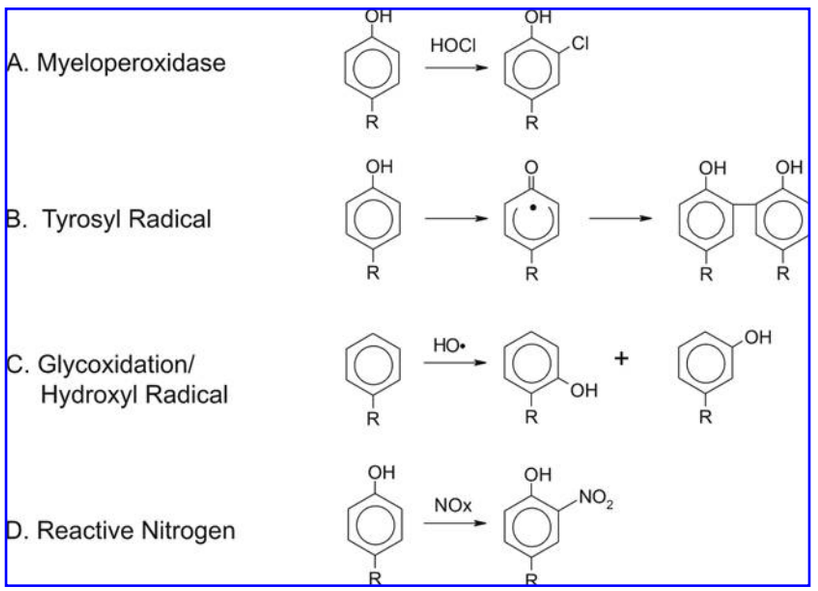

FIG. 2. Proposed oxidation products of protein-bound aromatic amino acids by myeloperoxidase, tyrosyl radical, glycoxidation/hydroxyl radical, and reactive nitrogen species (RNS). Myeloperoxidase converts tyrosine to 3-chlorotyrosine; tyrosyl radical cross-links tyrosine to form o,o´-dityrosine; RNS convert tyrosine to 3-nitrotyrosine; hydroxyl radical produces *ortho*-tyrosine and *meta*-tyrosine from phenylalanine.

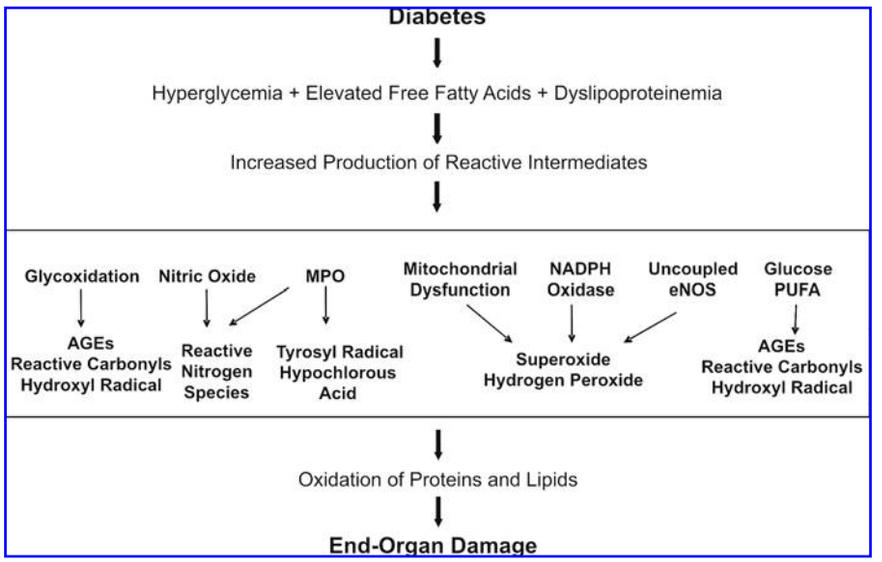

FIG. 3. Potential pathways for increased oxidant generation in diabetes and atherosclerosis. AGE, advanced glycosylation end-products; eNOS, endothelial nitric oxide synthase; MPO, myeloperoxidase.

AGEs can damage tissues through a number of mechanisms, including generation of oxidizing intermediates, formation of immune complexes, interaction with a cellular receptor called RAGE (Receptor for AGE), and promotion of cytokine release (5, 131). Although RAGE binds to AGE-modified proteins *in vitro* with high affinity, its ligands *in vivo* are unclear. High levels of AGEs accumulate in renal failure, even in nondiabetic patients, and this process reverses after renal transplantation, implicating the kidneys in AGE production and/or clearance (6, 92–94).

Many studies have shown that age-adjusted levels of pentosidine and N^{ϵ}-carboxymethyllysine (CML), two widely investigated AGE products, correlate with the development of diabetic complications (34, 63, 89, 95, 121). Thus, glycoxidation reactions can be one mechanism for diabetic complications.

The reactive nitrogen pathway. Another pathway for generating oxidants involves nitric oxide (NO), which is produced by endothelial cells to regulate vascular tone. NO is also produced during inflammation by macrophages, which are early components of atherosclerotic lesions. NO reacts with superoxide ($O_2^{\bullet-}$) to generate peroxynitrite ($ONOO^-$; Equation 2), a potent oxidant that converts tyrosine residues to 3-nitrotyrosine.

$$O_2^{\bullet-} + NO^{\bullet} \rightarrow ONOO^- \qquad \text{(Eq. 2)}$$

Thus, 3-nitrotyrosine is a marker for the reactive nitrogen pathway. It has been detected in low-density and high-density lipoproteins (LDL and HDL) isolated from human diabetic atherosclerotic lesions (83, 84, 107), and plasma nitrotyrosine levels are elevated in patients with CAD (125, 126). Because acute hyperglycemia promotes vasodilation in humans, glucose might directly or indirectly enhance NO release and oxidant generation (122).

The myeloperoxidase pathway. The major pathway through which macrophages and other phagocytic cells of the innate immune system generate oxidants begins with the cells' membrane-bound NADPH oxidase (NOX), which produces superoxide (Equation 3).

$$NADPH + 2\ O_2 \rightarrow NADP^+ + H^+ + 2\ O_2^{\bullet-} \qquad \text{(Eq. 3)}$$

Superoxide dismutates into hydrogen peroxide (Equation 4).

$$2\ H^+ + 2\ O_2^{\bullet-} \rightarrow H_2O_2 + O_2 \qquad \text{(Eq. 4)}$$

The peroxide can be used by another phagocyte enzyme, myeloperoxidase (77, 78), to convert chloride ion to hypochlorous acid (Equation 5).

$$H_2O_2 + Cl^- + H^+ \rightarrow HOCl + H_2O \qquad \text{(Eq. 5)}$$

Oxidation of NO with oxygen yields nitrite (NO_2^-), which myeloperoxidase converts to nitrogen dioxide radical ($NO_2^{\bullet}$; Equation 6), a potent nitrating intermediate (37, 40).

$$NO_2^- + H_2O_2 \rightarrow NO_2^{\bullet} + H_2O \qquad \text{(Eq. 6)}$$

Reactive nitrogen species, including peroxynitrite and $NO_2^{\bullet}$, might contribute to the inflammatory process by nitrating lipoproteins and other biomolecules.

Hyperglycemia can activate PKC (70, 71, 75), which leads to phagocyte activation, secretion of myeloperoxidase, and oxidant generation. NEFAs that commonly are overabundant in diabetes can also activate phagocytes *in vitro*. These changes might enhance the production of superoxide and hydrogen peroxide, which myeloperoxidase converts into more potent cytotoxic oxidants, such as hypochlorous acid and nitrogen dioxide radical.

The mitochondrial pathway. Mitochondrial electron transport generates superoxide. Glucose promotes mitochondrial electron transport in cells, and a glucose-driven mitochondrial oxidation pathway has been proposed as one

mechanism for damaging cells in diabetes (16). When cultured endothelial cells are exposed to high levels of glucose, their mitochondria increase their superoxide output (33). Endothelial cells transfected with mitochondrial superoxide dismutase, a scavenger of superoxide, do not show this response. Thus, superoxide production by the mitochondrial electron transport chain could be one mechanism for diabetic tissue damage (16, 101). The mitochondrial pathway can be blocked by inhibitors of oxidative phosphorylation (16) or by a superoxide dismutase mimic (118).

Mitochondrial superoxide overproduction mediated by hyperglycemia might also increase polyol pathway activity, PKC activity, and hexosamine flux, resulting in cellular dysfunction and tissue damage (16). Moreover, superoxide inhibits glyceraldehyde phosphate dehydrogenase, a key glycolytic enzyme whose inactivity could make upstream metabolites accumulate. Such inhibition of glycolysis might promote end-organ damage by diverting metabolites into the hexosamine pathway or stimulating the polyol and diacylglycerol-PKC pathways. Benfotiamine, a lipid-soluble thiamine analog that inhibits these pathways by activating transketolase, an enzyme in the pentose pathway shunt, can prevent complications from experimental diabetes in animal models (48).

Endothelial cell dysfunction and excess superoxide production. Endothelial dysfunction has been proposed to generate excess superoxide, resulting in oxidative stress**.** Two enzymes have been implicated: NOX and uncoupled endothelial NO synthase (eNOS). Glucose and NEFAs can directly stimulate NOX because they activate PKC (69). Apocynin, which inhibits both NOX and PKC, reduced vascular superoxide generation in an animal model of type 1 diabetes and two models of type 2 diabetes (obese ob/ob mice and Zucker fatty rats) (23, 128). Additionally, it has been demonstrated that AGEs (138), oxidized LDL (72), and angiotensin II (25) can also increase NOX activity. Angiotensin II may represent a pathophysiologically relevant pathway for stimulating the production of reactive intermediates by artery wall cells because inhibitors of this pathway lower the risk for cardiovascular events in diabetics (60).

Oxidation of tetrahydrobiopterin, the reduced form of biopterin that is required for arginine reduction and NO production by eNOS, uncouples eNOS from NO synthesis. Under these conditions, eNOS transfers electrons to molecular oxygen to generate superoxide (136). An alternative mechanism for uncoupling eNOS involves angiotensin II, which can induce dihydrofolate reductase deficiency, perhaps via a hydrogen peroxide-dependent mechanism. Because dihydrofolate reductase maintains tetrahydrobiopterin in its reduced form, dihydrofolate reductase deficiency results in eNOS uncoupling (25). Recently, it has been demonstrated that attenuation of angiotensin II signaling can recouple eNOS and inhibit peroxide production by NOX in diabetic mice (102).

The glucose-polyunsaturated fatty acid (PUFA) pathway. To determine whether glucose can generate reactive intermediates by interacting with polyunsaturated fatty acids, we incubated it with LDL or a model protein, RNase (109). The buffer was free of redox-active metal ions because it had been treated with Chelex resin and supplemented with a metal chelator. Under these conditions, pathophysiologically relevant concentrations of glucose modified LDL, as evidenced by the formation of oxidized amino acids, even though metal ions were absent (Fig. 4). In striking contrast, glucose exposure did not increase levels of oxidized amino acids in RNase. These observations suggest that glucose promotes LDL oxidation because the particle contains lipid as well as protein. Our observation that levels of lipid oxidation products rise when LDL is exposed to glucose is consistent with this proposal.

To test the hypothesis that lipid can enhance protein oxidation, we incubated RNase with saturated, monounsaturated, or PUFA (Fig. 5). Glucose stimulated protein oxidation only in the presence of a PUFA. Thus, glucose appears to promote protein oxidation by a pathway involving peroxidation of PUFAs as this reaction is inhibited by lipid-soluble antioxidants (109).

To determine whether glucose's carbonyl group promotes LDL oxidation, we replaced glucose with a variety of short-chain and phosphorylated sugars that have highly reactive carbonyl groups (109). All of the carbonyl compounds promoted oxidation of LDL (but not RNASe) protein more effectively than did glucose. In contrast, LDL oxidation was not enhanced by sorbitol, the reduced form of glucose that lacks a carbonyl moiety (Fig. 6). Collectively, these observations indicate that glucose can generate a species resembling the hydroxyl radical by a carbonyl/PUFA pathway. This pathway might therefore promote localized oxidative stress in tissues vulnerable to diabetic damage (109).

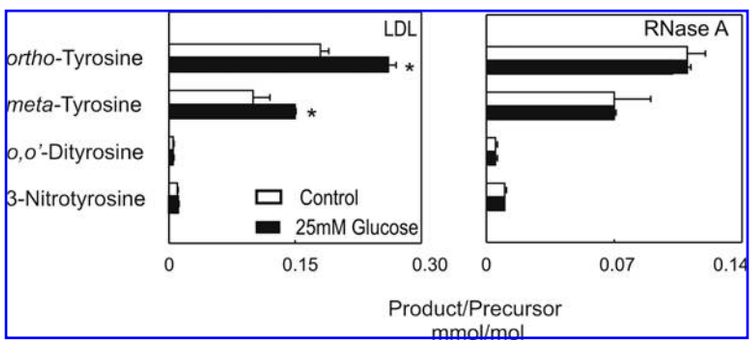

FIG. 4. Product yields of *ortho*-tyrosine, *meta*-tyrosine, *o,o´*-dityrosine, and 3-nitrotyrosine in LDL and RNase exposed to D-glucose *in vitro*. LDL and RNase (1 mg protein/ml of buffer) were incubated with 0 m*M* (Control) or 25 m*M* D-glucose. At the end of the incubation, amino acids in the protein were quantified by GC/MS. **p* < 0.05 by Student's *t*-test. Reproduced from (109).

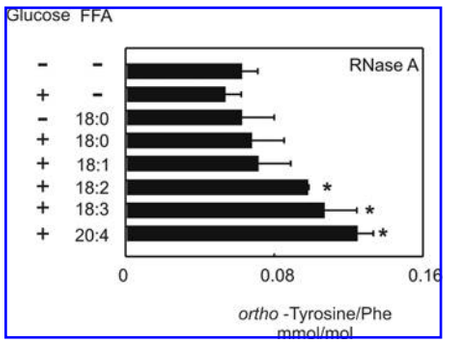

FIG. 5. Reaction requirements for oxidation of RNase by D-glucose. RNase was incubated in buffer with and/or without 25 m*M* of fatty acid (stearate, 18:0; oleate, 18:1; linoleate, 18:2; linolenate, 18:3; arachidonate, 20:4) and/or 25 m*M* D-glucose. At the end of the incubation, amino acids in the protein were quantified by GC/MS. *$p < 0.05$ by Student's *t*-test. Reproduced from (109).

OXIDIZED AMINO ACIDS SERVE AS MOLECULAR FINGERPRINTS FOR SPECIFIC OXIDATION PATHWAYS

The examples given above illustrate that many different pathways can oxidize lipoproteins *in vitro*. To understand the molecular mechanisms that promote oxidative stress *in vivo,* we first used a chemical approach to define the patterns of oxidation products that are formed by well-characterized oxidant-generating systems *in vitro*. Then we looked for similar patterns in tissue and plasma. We focused on proteins because aromatic amino acids retain the initial footprint of the reactive intermediate that initiates oxidative damage. In contrast, lipid peroxidation products readily undergo subsequent chain-propagating reactions, which mask the products formed early in the pathway. Moreover, many different oxidizing intermediates give the same spectrum of oxidized lipids, making it difficult to identify specific pathways that trigger lipid oxidation.

Oxidizing intermediates are difficult to detect *in vivo* because they are short-lived and generated at low levels. To sidestep this problem, we and others have identified and are able to monitor acid-stable products of protein oxidation, both *in vitro* and *in vivo* (2, 5, 7–9, 12, 13, 37, 51, 56, 57, 80–84, 107–111). Our overall approach is to use isotope dilution GC/MS to accurately identify oxidized amino acids isolated from tissue proteins. These markers, which include *ortho*-tyrosine, *meta*-tyrosine, dityrosine, 3-nitrotyrosine, and 3-chlorotyrosine (Fig. 2), indicate which biochemical pathway has damaged a protein.

Evidence for localized oxidative stress in diabetes

Indirect evidence supports the hypothesis that oxidative stress increases in diabetes. For example, numerous investigators have reported elevated levels of products of lipid, protein, and nucleic acid oxidation in the blood of diabetic patients, though most of these products were monitored with nonspecific assays that may not yield valid results with complex biological material (6). Studies using sensitive and specific MS methods to quantify oxidation products have cast doubt on the concept of a generalized increase in oxidative stress in diabetic humans. For example, Wells–Knecht *et al.* (141) performed careful, quantitative studies on collagen, a long-lived protein that is freely exposed to blood glucose and lipids. They concluded that diabetes does not enhance oxidative stress because collagen from diabetic and euglycemic subjects contained similar age-adjusted levels of *ortho*-tyrosine and methionine sulfoxide, two well-characterized markers of protein oxidation *in vitro*. Other mass spectrometric studies have failed to find differing levels of glycoxidation products in urine and blood of diabetic and euglycemic humans (6, 35). These observations argue strongly against a generalized

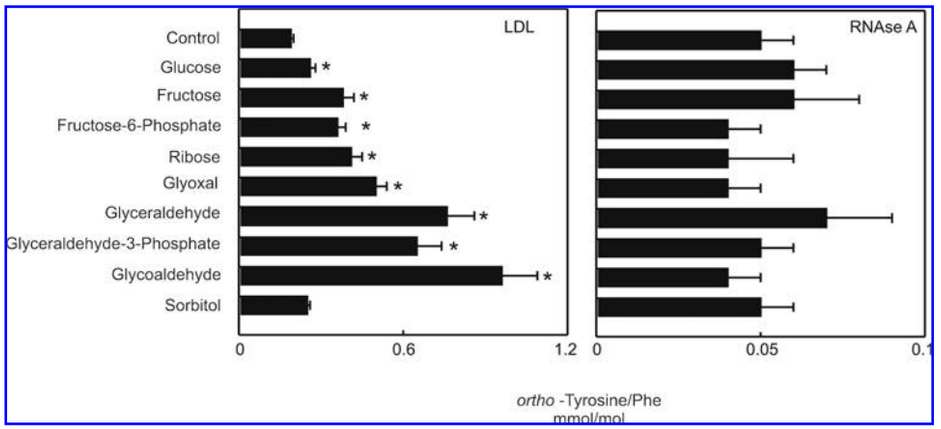

FIG. 6. Quantification of *ortho*-tyrosine in LDL and RNase incubated with reactive carbonyl compounds and sorbitol. LDL or RNase was incubated in buffer supplemented with 25 m*M* of the indicated sugar for 15 days at 37°C. At the end of the incubation, amino acids in the proteins were quantified by GC/MS. *$p < 0.05$ by Student's *t*-test. Reproduced from (109).

increase in oxidative stress in diabetes, at least in the extracellular compartment. None of the above studies excluded the possibility of localized, tissue-specific increases in oxidative stress in organs vulnerable to diabetic damage: the retina, kidney, vascular wall, and peripheral nerve tissue.

MS quantitation of oxidized amino acids in aortic proteins of diabetic Cynomolgus monkeys reveals localized oxidative damage by hydroxyl radical

To investigate the potential role of localized oxidative stress in diabetic macrovascular disease, we used a primate model of diabetic atherosclerosis. These studies involved *Cynomolgus* monkeys that had been hyperglycemic for 6 months due to streptozotocin-induced diabetes (111). We analyzed samples from 7 controls and 8 diabetic *Cynomolgus* monkeys, sampling three different areas from the thoracic aorta of each animal. Compared with the control samples, the aorta wall proteins from the diabetic animals showed a significant 40% increase in *ortho*-tyrosine (Fig. 7A) and a similar (60%) increase in *meta*-tyrosine (Fig. 7B). *In vitro* studies identify these oxidized amino acids as products of hydroxyl radical, implicating a hydroxyl radical-like species in damage to artery wall proteins in diabetic monkeys.

The concentration of *o,o´*-dityrosine, a marker of tyrosyl radical that is produced in lower yield by hydroxyl radical (Fig. 7C), was fourfold greater in the diabetic samples than in the control samples. Thus, *ortho*-tyrosine, *meta*-tyrosine, and *o,o´*-dityrosine levels were higher in aortic proteins from diabetic monkeys than in those from control animals. However, we found no significant difference in 3-nitrotyrosine levels in proteins isolated from aortic tissue of control and diabetic *Cynomolgus* monkeys (Fig. 7D). Collectively, these results indicate that *ortho*-tyrosine, *meta*-tyrosine, and *o,o´*-dityrosine levels are selectively elevated in aortic proteins of diabetic monkeys, whereas 3-nitrotyrosine levels remain unchanged. This pattern of oxidized amino acids suggests that a hydroxyl-radical like oxidant promotes aortic damage in this animal model of type 1 diabetes.

Correlations between aortic tissue levels of the oxidized amino acids and serum levels of glycated hemoglobin

To determine whether glucose promotes protein oxidation *in vivo*, we assessed the relationship between level of glycemic control (measured as serum glycated hemoglobin) and levels of amino acid oxidation products in aortic tissue in control and diabetic *Cynomolgus* monkeys (Fig. 8). Linear regression analysis demonstrated a strong correlation between levels of both *ortho*-tyrosine and *meta*-tyrosine and glycated hemoglobin ($r^2 = 0.9$ and 0.8, respectively; both $p < 0.001$). Levels of *o,o´*-dityrosine and glycated hemoglobin correlated less strongly ($r^2 = 0.3$; $p = 0.07$). In contrast, there was no correlation between levels of 3-nitrotyrosine and glycated hemoglobin. These observations support the hypothesis that glucose promotes the formation of *ortho*-tyrosine and *meta*-tyrosine in the artery wall and suggest that both glucose and other pathways contribute to the generation of *o,o´*-dityrosine (111).

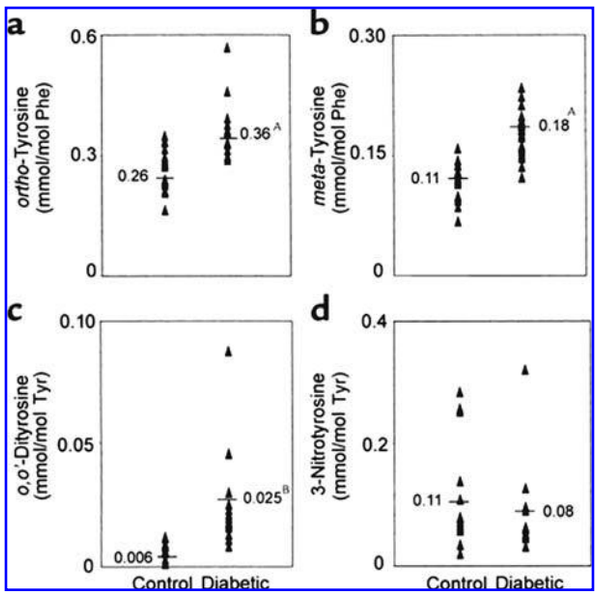

FIG. 7. Quantification of *ortho*-tyrosine (a), *meta*-tyrosine (b), *o,o´*-dityrosine (c), and 3-nitrotyrosine (d) in aortic proteins isolated from control and diabetic *Cynomolgus* monkeys. Aortic tissue was harvested from control and diabetic animals at the end of the 6-month study. Tissue was delipidated, hydrolyzed, and amino acids were quantified by GC/MS. *$p < 0.01$ by Student's *t*-test. Reproduced from (111).

Plasma and urinary levels of oxidized amino acids are potential markers for assessing oxidative stress in vivo

There is increasing evidence that oxidized amino acids in plasma and urine can serve as markers for noninvasive

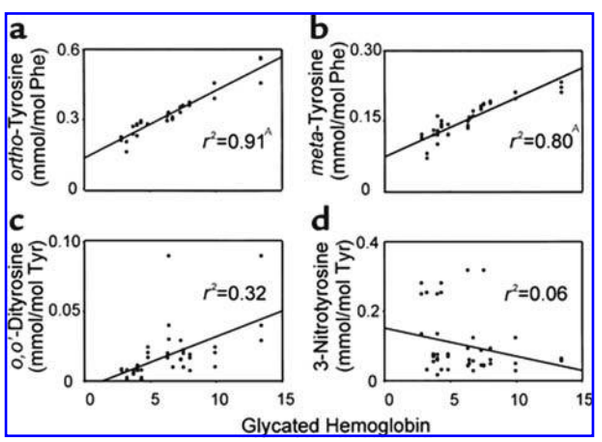

FIG. 8. Concentrations of *ortho*-tyrosine (a), *meta*-tyrosine (b), *o,o´*-dityrosine (c), and 3-nitrotyrosine (d) in aortic proteins of *Cynomolgus* monkeys as a function of glycated hemoglobin. Lines represent the linear least squares fit of the data. Glycated hemoglobin was determined in serum of both control and diabetic animals at the end of the 6-month study. *$p < 0.001$ by Spearman rank correlation. Reproduced from (111).

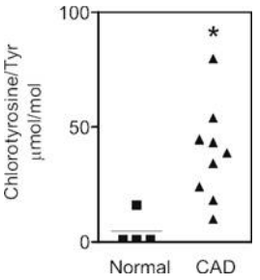

FIG. 9. MS quantification of 3-chlorotyrosine in HDL isolated from plasma. Plasma was obtained from healthy control subjects and subjects with established CAD. Oxidized amino acids isolated from hydrolyzed HDL proteins were quantified by GC/MS. $p < 0.01$ by Student's *t*-test. Reproduced from (8).

assessment of oxidative stress *in vivo*. Plasma and urinary levels of these markers are likely to be proportional to the rate of generation and thus can serve as indices of chronic oxidative stress *in vivo* (8, 9, 82, 107, 108, 125–127, 150).

To determine whether oxidized amino acids in urine might serve as markers of oxidative stress, we determined the urinary excretion of *o,o´*-dityrosine in a mouse model of peritoneal inflammation (9). To correct for individual differences in glomerular filtration, we normalized *o,o´*-dityrosine levels to those of urinary creatinine. The concentration of *o,o´*-dityrosine was three-fold higher in the urine of mice with active peritoneal inflammation, but it failed to increase in mice lacking phagocytic gp91^{phox} subunit of NOX. These results suggest that degradation of oxidatively damaged proteins in phagocytes releases free *o,o´*-dityrosine that enters the blood and is excreted in urine.

These observations may be relevant to human pathophysiology. For example, a case-control study demonstrated that systemic levels of protein-bound nitrotyrosine were significantly higher in patients with CAD than in controls with healthy arteries. Moreover, statin therapy lowered levels of oxidation markers in plasma, raising the possibility that these drugs can potentially be antioxidants (125–127). Therefore, these markers might serve not only to assess oxidative stress but also to monitor the efficacy of therapy.

Oxidized HDL is a plasma marker of oxidative stress in subjects with CAD

Because lipoprotein oxidation is implicated in atherogenesis, we analyzed plasma lipoproteins for oxidized amino acids. Preliminary studies demonstrated that HDL, but not LDL, isolated from plasma of subjects with established CAD contained high levels of 3-chlorotyrosine, a highly specific marker for the myeloperoxidase pathway. The level of 3-chlorotyrosine was 13-fold higher in HDL isolated from plasma of subjects with established CAD than in HDL from plasma of healthy subjects (Fig. 9); (8). We also found that levels of 3-nitrotyrosine were twice as high in HDL from plasma of patients with established CAD (Fig. 10); (107)). These observations raise the exciting possibility that oxidized HDL might be a novel marker for clinically significant CAD. It will clearly be important to determine whether levels of oxidized amino acids are elevated in HDL and LDL of diabetic humans.

Lipoxidation in diabetes mellitus

Several observations suggest a complex interplay among protein oxidation, lipid peroxidation, and AGE formation. When LDL is incubated with glucose or glycated proteins, lipoprotein oxidation increases (18, 19, 66, 74). Moreover, CML, an extensively studied AGE, forms from PUFAs during lipid peroxidation (39). Thus, CML is a product of both glycoxidation and lipoxidation. CML and related glycoxidation products can form not only from early intermediates in the Maillard reaction but also directly from glyoxal or methylglyoxal, which may in turn be derived from either carbohydrates or lipids (6). PUFAs are oxidized much more readily than is glucose, and dyslipidemia is common in diabetic patients. Thus, elevated levels of plasma lipoproteins might contribute to lipoxidation *in vivo*. AGE lipids have also been found in diabetic plasma lipoproteins (18, 19, 114–116), and levels of isoprostanes—specific markers of nonenzymatic lipoxidation—are elevated in diabetic patients (28, 91). CML and other AGE compounds could thus originate *in vivo* when carbohydrates or lipids react with proteins.

Aminoguanidine and pyridoxamine comprise of a new class of agents termed "amadorins" that prevent the amadori product from forming AGEs. Both inhibit AGE formation from carbohydrates, and pyridoxamine also inhibits advanced lipoxidation reactions (104, 137). Aminoguanidine prevents diabetic complications in animal models, which may reflect its ability to inhibit AGE formation and inducible nitric oxide synthase (17, 36, 47, 49, 68). Clinical trials were halted, however, because aminoguanidine proved too toxic to humans. Pyridoxamine is effective in preventing retinopathy and nephropathy in diabetic rats (31, 132), but its effects on humans remain to be determined.

Carbonyl stress in diabetes

Glucose is a reactive carbonyl that can generate additional carbonyls and other reactive compounds through both enzymatic and nonenzymatic reactions. Baynes and Thorpe proposed that diabetes is a characterized by carbonyl stress that in turn may account for the increased protein and lipid modifications that typify diabetes and uremia (6). In this model, reactive carbonyl production rises due to increased substrate stress (elevated levels of glucose and its derivatives), and is compounded by deficiency or overload of pathways that detoxify carbonyl compounds. The abundance of reactive carbonyls enhances protein and lipid modification. Oxidative stress is thus a primary event that increases carbonyl production, but is also a secondary event due to elevated carbonyl levels (6).

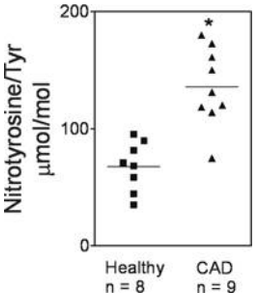

FIG. 10. MS quantification of 3-nitrotyrosine in HDL isolated from plasma. Plasma was obtained from healthy control subjects and subjects with established CAD. Oxidized amino acids isolated from hydrolyzed HDL proteins were quantified by GC/MS $*p < 0.01$ by Student's *t*-test. Reproduced from (107).

Glucose promotes lipid and protein oxidation in diabetic retinal tissue by a carbonyl- and PUFA-dependent pathway

To begin to explore the pathophysiological relevance of the carbonyl-PUFA pathway, we quantified levels of protein and lipid oxidation products in the retina of hyperglycemic Sprague–Dawley rats, a well-characterized model of diabetic retinopathy (109). Abnormalities such as increased vascular permeability became well established in retinal tissue as early as 4 weeks after STZ treatment (85, 149). After 6 weeks, retinal tissue contained markedly elevated levels of *ortho*-tyrosine and *meta*-tyrosine and high levels of hydroxy-octadecadienoic acid, a major product of lipid oxidation. Treatment with the carbonyl scavenger aminoguanidine blocked these changes but failed to affect levels of *ortho*- and *meta*-tyrosine in control animals. We observed no differences in levels of D-glucose or hemoglobin A_1C in the rats on the two different diets. Thus, glucose or other reactive carbonyls that abound in the diabetic milieu promote lipid and protein oxidation *in vivo* by a pathway requiring PUFAs. These observations suggest that glucose might operate locally by a variety of mechanisms, damaging artery wall proteins even in the absence of generalized oxidative stress. It will be important to determine whether the pattern of oxidation products in diabetic humans resembles the ones we observed in the monkey and rat studies.

Hyperlipidemia in concert with hyperglycemia stimulates the proliferation of macrophages in atherosclerotic lesions: potential role of glucose-oxidized LDL

Macrophage proliferation has been implicated in the progression of atherosclerosis. Recent studies have investigated the effects of hyperglycemia and hyperlipidemia on macrophage proliferation in murine atherosclerotic lesions and isolated primary macrophages (80). Glucose promoted lipid and protein oxidation of LDL *in vitro*. Oxidation of LDL with glucose resulted in a selective increase in protein-bound *ortho*-tyrosine and *meta*-tyrosine. Moreover, glucose-oxidized LDL—but not elevated levels of glucose alone—stimulated proliferation of isolated macrophages. These observations may be pertinent to diabetic vascular disease because macrophage proliferation in atherosclerotic lesions was observed in LDL receptor-deficient mice that were both hypercholesterolemic and hyperglycemic but in not mice that were only hyperglycemic (80).

Antioxidants for preventing diabetic complications

The proposed role of oxidized LDL in atherogenesis suggests that a high dietary antioxidant intake might prevent premature vascular disease in humans. However, the majority of prospective, double-blind, placebo-controlled trials of one proposed lipid-soluble antioxidant, vitamin E, have failed to demonstrate any reduction of clinical events in patients with established atherosclerosis (54). The disappointing results of these trials have led many to question the role of oxidative damage in the pathogenesis of coronary artery disease in humans. It might be, however, that vitamin E's ability to serve as an antioxidant *in vivo* should be questioned (54). Thus, despite the impressive ability of other lipid-soluble antioxidants to block atherosclerosis in hypercholesterolemic animals, vitamin E—at doses that fail to lower cholesterol levels—has not exerted a consistent inhibitory effect in such experiments. These observations (53, 54, 87) emphasize the importance of documenting that a proposed antioxidant intervention actually inhibits oxidative reactions *in vivo*.

There is also remarkably little information about the influence of vitamin E supplementation on lipid oxidation in humans. Indeed, a recent study of healthy humans taking dietary supplements as high as 2,000 IU/day for 8 weeks found no change in levels of three lipid oxidation products: 4-hydroxynonenal and two isoprostanes (90). The investigators assessed products of lipid peroxidation using GC/MS, a sensitive and specific method. These results strongly suggest that vitamin E failed to inhibit lipid peroxidation in these individuals.

We have shown that one pathway for LDL oxidation in the human artery wall involves myeloperoxidase (27, 55). It is therefore noteworthy that vitamin E also fails to inhibit many of the reactions executed by that enzyme, that myeloperoxidase and its characteristic products are found at high levels in human atherosclerotic tissue, and that myeloperoxidase oxidizes LDL by reactions that are independent of lipid peroxidation (50, 52, 58, 119). These observations highlight the importance of identifying both the targets of oxidation in the artery wall and the biochemical pathways that promote oxidative reactions *in vivo*, using sensitive and specific analytical techniques such as MS.

Trials of antioxidants and carbonyl trapping agents in humans suffering from diabetes have also yielded discouraging results. Chronic treatment with vitamin E failed to decrease cardiovascular events in a large study that included a high percentage of diabetic patients (148). One possible reason is that antioxidant therapy might benefit only subjects who exhibit increased oxidative stress. Indeed, the renal failure patients who benefited from vitamin E therapy (10) might have been a subset with greatly increased carbonyl and oxidative stress (94).

Certain inhibitors of oxidant generation and proposed antioxidants have shown promise in preliminary studies of diabetic neuropathy. These include the xanthine oxidase inhibitor, allopurinol, and the chain-breaking antioxidant alpha-lipoic acid (ALA). A recent report (21) examined the effects of allopurinol on endothelial function and oxidative stress in type 2 diabetic patients. The investigators showed that allopurinol increased the endothelium-dependent mean blood flow response to acetylcholine by 30% and decreased systemic levels of malondialdehyde. However, the relevance of endothelium-dependent mean blood flow response as a surrogate marker for cardiovascular disease has been questioned because this technique predicted that vitamin E would prevent vascular disease.

ALA is an endogenous free radical scavenger (97, 105) and metal chelator (105). ALA is reduced metabolically to dihydrolipoate, which combines free radical scavenging and metal chelating properties with the ability to regenerate high concentrations of nonenzymatic and enzymatic antioxidants (86,

103, 105). In the Alpha-Lipoic Acid in Diabetic Neuropathy (ALADIN) Study, neuropathic symptoms lessened in type 2 diabetic subjects treated with intravenous ALA for 3 weeks (152). In the ALADIN III Study, 509 subjects with type 2 diabetes and symptomatic diabetic neuropathy were administered sequential treatment with 600 mg/day ALA intravenously for 3 weeks followed by 600 mg thrice daily orally for 6 months. The total symptom score for neuropathy symptoms was significantly better at early time points in the ALA-treated group and neuropathic deficits were consistently improved throughout the study (151). In the Deutsche Kardiale Autonome Neuropathie (DEKAN) Study, heart rate variability improved after 4 months of oral treatment with ALA (800 mg/day) in type 2 diabetic patients with neuropathy (153). Whether long-term oral therapy with ALA will be effective in diabetic neuropathy remains to be established. The clinical significance of affecting heart rate variability in diabetics is also unclear.

Pharmacologic agents currently in clinical practice with demonstrated benefit in CAD may act in part by serving as antioxidants. Angiotensin converting enzyme (ACE) inhibitors and statins have lowered CAD event rates in randomized controlled trials in diabetic patients (26, 60). As noted above, many lines of evidence suggest that angiotensin II triggers oxidant production by endothelial cells and other cells of the artery wall. Interestingly, the ACE inhibitor ramipril slowed the onset of type 2 diabetes in the Heart Outcomes Prevention Evaluation (HOPE) trial (60), though this effect was not confirmed in a more recent trial (11). Ramipril's well-recognized ability to mitigate the pro-oxidant effect of angiotensin II may in part account for its efficacy. A case-control study demonstrated that systemic levels of protein-bound nitrotyrosine were significantly higher among patients with CAD and that statin therapy lowered levels of oxidized amino acids in plasma, raising the possibility that statins can potentially be antioxidants (125–127). Thiazolidinediones (TZD), which have been proposed to improve endothelial dysfunction in part by decreasing oxidative stress via activation of the peroxisome-proliferator-activated receptor γ, have now been shown to decrease carotid intimal thickness in diabetic subjects independently of their ability to boost glycemic control (88). Additionally, TZD treatment also decreased progression to overt diabetes in subjects with impaired glucose tolerance (32). The oxidative pathways modulated by these agents have not been defined and should be the focus of future investigations.

CONCLUSIONS AND PERSPECTIVES

The ability to accurately quantify amino acid oxidation markers in tissue samples, plasma, and urine can provide invaluable mechanistic insights into disease pathogenesis. Therefore, further studies of the biochemical basis for oxidant generation might facilitate the development of specific antioxidant therapies designed to retard diabetic complications.

The potential role of antioxidant therapies in preventing microvascular and macrovascular disease in diabetic humans is another important issue. To examine the oxidative stress hypothesis in diabetes, suitable test subjects and optimal antioxidant regimens need to be identified. Such a trial should involve diabetic patients with evidence of increased oxidative stress (for example, subjects with elevated levels of oxidized amino acids in their plasma or urine). These high-risk individuals are most likely to benefit from antioxidant therapy. The optimal regimen for inhibiting oxidative tissue injury in humans also needs to be determined so that test compounds can be administered in effective antioxidant regimens.

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ABBREVIATIONS

ACE, angiotensin converting enzyme; AGEs, advanced glycation end products; ALA, alpha-lipoic acid; CML, N^{ϵ}-(carboxymethyl)lysine; eNOS, endothelial nitric oxide synthase; GC/MS, gas chromatography–mass spectrometry; HDL, high density lipoprotein; LDL, low density lipoprotein; NEFA, nonesterified fatty acid; NO, nitric oxide; NOX, NADPH oxidase; PKC, protein kinase C; PUFA, polyunsaturated fatty acid; RBP4, retinol-binding protein 4; TZD, thiazolidinedione.

REFERENCES

1. Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, and Kahn BB. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature* 409: 729–733, 2001.
2. Abu–Soud HM and Hazen SL. Nitric oxide is a physiological substrate for mammalian peroxidases. *J Biol Chem* 275: 37524–37532, 2000.
3. American Diabetes Association. Diabetes Statistics. *http://wwwdiabetesorg/diabetes-statisticsjsp*, 2007. (Accessed January 28, 2007.)
4. Ames BN, Shigenaga MK, and Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. *Proc Natl Acad Sci USA* 90: 7915–7922, 1993.
5. Baynes JW and Thorpe SR. Glycoxidation and lipoxidation in atherogenesis. *Free Radic Biol Med* 28: 1708–1716, 2000.
6. Baynes JW and Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* 48: 1–9, 1999.
7. Beckman JS, Chen J, Ischiropoulos H, and Crow JP. Oxidative chemistry of peroxynitrite. *Methods Enzymol* 233: 229–240, 1994.
8. Bergt C, Pennathur S, Fu X, Byun J, O'Brien K, McDonald TO, Singh P, Anantharamaiah GM, Chait A, Brunzell J, Geary RL, Oram JF, and Heinecke JW. The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. *Proc Natl Acad Sci USA* 101: 13032–13037, 2004.

9. Bhattacharjee S, Pennathur S, Byun J, Crowley J, Mueller D, Gischler J, Hotchkiss RS, and Heinecke JW. NADPH oxidase of neutrophils elevates o,o'-dityrosine cross-links in proteins and urine during inflammation. *Arch Biochem Biophys* 395: 69–77, 2001.
10. Boaz M, Smetana S, Weinstein T, Matas Z, Gafter U, Iaina A, Knecht A, Weissgarten Y, Brunner D, Fainaru M, and Green MS. Secondary prevention with antioxidants of cardiovascular disease in end stage renal disease (SPACE): randomised placebo controlled trial. *Lancet* 356: 1213–1218, 2000.
11. Bosch J, Yusuf S, Gerstein HC, Pogue J, Sheridan P, Dagenais G, Diaz R, Avezum A, Lanas F, Probstfield J, Fodor G, and Holman RR. Effect of ramipril on the incidence of diabetes. *N Engl J Med* 355: 1551–1562, 2006.
12. Brennan ML and Hazen SL. Emerging role of myeloperoxidase and oxidant stress markers in cardiovascular risk assessment. *Curr Opin Lipidol* 14: 353–359, 2003.
13. Brennan ML, Penn MS, Van Lente F, Nambi V, Shishehbor MH, Aviles RJ, Goormastic M, Pepoy ML, McErlean ES, Topol EJ, Nissen SE, and Hazen SL. Prognostic value of myeloperoxidase in patients with chest pain. *N Engl J Med* 349: 1595–1604, 2003.
14. Brown MS and Goldstein JL. Koch's postulates for cholesterol. *Cell* 71: 187–188, 1992.
15. Brownlee M. Advanced protein glycosylation in diabetes and aging. *Annu Rev Med* 46: 223–234, 1995.
16. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 414: 813–820, 2001.
17. Brownlee M, Vlassara H, Kooney A, Ulrich P, and Cerami A. Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science* 232: 1629–1632, 1986.
18. Bucala R, Makita Z, Koschinsky T, Cerami A, and Vlassara H. Lipid advanced glycosylation: pathway for lipid oxidation *in vivo. Proc Natl Acad Sci USA* 90: 6434–6438, 1993.
19. Bucala R, Makita Z, Vega G, Grundy S, Koschinsky T, Cerami A, and Vlassara H. Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency. *Proc Natl Acad Sci USA* 91: 9441–9445, 1994.
20. Bucala R and Vlassara H. Advanced glycosylation end products in diabetic renal and vascular disease. *Am J Kidney Dis* 26: 875–888, 1995.
21. Butler R, Morris AD, Belch JJ, Hill A, and Struthers AD. Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. *Hypertension* 35: 746–751, 2000.
22. Carew TE, Schwenke DC, and Steinberg D. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Proc Natl Acad Sci USA* 84: 7725–7729, 1987.
23. Cave AC, Brewer AC, Narayanapanicker A, Ray R, Grieve DJ, Walker S, and Shah AM. NADPH oxidases in cardiovascular health and disease. *Antioxid Redox Signal* 8: 691–728, 2006.
24. Centers for Disease Control and Prevention**.** National diabetes fact sheet: general information and national estimates on diabetes in the United States, 2003. *US Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta GA*, 2004.
25. Chalupsky K and Cai H. Endothelial dihydrofolate reductase: critical for nitric oxide bioavailability and role in angiotensin II uncoupling of endothelial nitric oxide synthase. *Proc Natl Acad Sci USA* 102: 9056–9061, 2005.
26. Collins R, Armitage J, Parish S, Sleigh P, and Peto R. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. *Lancet* 361: 2005–2016, 2003.
27. Daugherty A, Dunn JL, Rateri DL, and Heinecke JW. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J Clin Invest* 94: 437–444, 1994.
28. Davi G, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, Pennese E, Vitacolonna E, Bucciarelli T, Costantini F, Capani F, and Patrono C. *In vivo* formation of 8-iso-prostaglandin f2alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. *Circulation* 99: 224–229, 1999.
29. DCCT Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329: 977–986, 1993.
30. 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* 342: 381–389, 2000.
31. Degenhardt TP, Alderson NL, Arrington DD, Beattie RJ, Basgen JM, Steffes MW, Thorpe SR, and Baynes JW. Pyridoxamine inhibits early renal disease and dyslipidemia in the streptozotocin-diabetic rat. *Kidney Int* 61: 939–950, 2002.
32. DREAM Trial Investigators. The Diabetes REduction Assessment with ramipril and rosiglitazone Medication (DREAM) trial. *Lancet* 368: 1096–1105, 2006.
33. Du XL, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, Wu J, and Brownlee M. Hyperglycemi-induced mitochondrial superoxide overproduction activates hexosamine pathway and induces plasminogen-activator inhibitor-1 expression by increasing Sp-1 glycosylation. *Proc Natl Acad Sci USA* 97: 12222–12226, 2000.
34. Dyer DG, Blackledge JA, Thorpe SR, and Baynes JW. Formation of pentosidine during nonenzymatic browning of proteins by glucose. Identification of glucose and other carbohydrates as possible precursors of pentosidine *in vivo*. *J Biol Chem* 266: 11654–11660, 1991.
35. Dyer DG, Dunn JA, Thorpe SR, Bailie KE, Lyons TJ, McCance DR, and Baynes JW. Accumulation of maillard reaction products in skin collagen in diabetes and aging. *J Clin Invest* 91: 2463–2469, 1993.
36. Edelstein D and Brownlee M. Aminoguanidine ameliorates albuminuria in diabetic hypertensive rats. *Diabetologia* 35: 96–97, 1992.
37. Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B, and van der Vliet A. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature* 391: 393–397, 1998.
38. Feldman EL. Oxidative stress and diabetic neuropathy: a new understanding of an old problem. *J Clin Invest* 111: 431–433, 2003.
39. Fu MX, Requena JR, Jenkins AJ, Lyons TJ, Baynes JW, and Thorpe SR. The advanced glycation end product, Nepsilon-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. *J Biol Chem* 271: 9982–9986, 1996.
40. Gaut JP, Byun J, Tran HD, Lauber WM, Carroll JA, Hotchkiss RS, Belaaouaj A, and Heinecke JW. Myeloperoxidase produces nitrating oxidants *in vivo*. *J Clin Invest* 109: 1311–1319, 2002.
41. Glass CK and Witztum JL. Atherosclerosis. the road ahead. *Cell* 104: 503–516, 2001.
42. Goldstein JL, Ho YK, Basu SK, and Brown MS. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc Natl Acad Sci USA* 76: 333–337, 1979.
43. Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ, Oberbach A, Jansson PA, Smith U, and Kahn BB. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N Engl J Med* 354: 2552–2563, 2006.
44. Haberland ME, Fong D, and Cheng L. Malondialdehyde-altered protein occurs in atheroma of Watanabe heritable hyperlipidemic rabbits. *Science* 241: 215–218, 1988.
45. Haffner SM. Coronary heart disease in patients with diabetes. *N Engl J Med* 342: 1040–1042, 2000.
46. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, and Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 339: 229–234, 1998.
47. Hammes HP, Brownlee M, Edelstein D, Saleck M, Martin S, and Federlin K. Aminoguanidine inhibits the development of

accelerated diabetic retinopathy in the spontaneous hypertensive rat. *Diabetologia* 37: 32–35, 1994.
48. Hammes HP, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, Lin J, Bierhaus A, Nawroth P, Hannak D, Neumaier M, Bergfeld R, Giardino I, and Brownlee M. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat Med* 9: 294–299, 2003.
49. Hammes HP, Martin S, Federlin K, Geisen K, and Brownlee M. Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. *Proc Natl Acad Sci USA* 88: 11555–11558, 1991.
50. Hazell LJ and Stocker R. Alpha-tocopherol does not inhibit hypochlorite-induced oxidation of apolipoprotein B-100 of low-density lipoprotein. *FEBS Lett* 414: 541–544, 1997.
51. Hazen SL. Myeloperoxidase and plaque vulnerability. *Arterioscler Thromb Vasc Biol* 24: 1143–1146, 2004.
52. Hazen SL and Heinecke JW. 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. *J Clin Invest* 99: 2075–2081, 1997.
53. Heinecke JW. Clinical trials of vitamin E in coronary artery disease: is it time to reconsider the low-density lipoprotein oxidation hypothesis? *Curr Atheroscler Rep* 5: 83–87, 2003.
54. Heinecke JW. Is the emperor wearing clothes? Clinical trials of vitamin E and the LDL oxidation hypothesis. *Arterioscler Thromb Vasc Biol* 21: 1261–1264, 2001.
55. Heinecke JW. Mass spectrometric quantification of amino acid oxidation products in proteins: insights into pathways that promote LDL oxidation in the human artery wall. *FASEB J* 13: 1113–1120, 1999.
56. Heinecke JW. Oxidative stress: new approaches to diagnosis and prognosis in atherosclerosis. *Am J Cardiol* 91: 12A–16A, 2003.
57. Heinecke JW. Oxidized amino acids: culprits in human atherosclerosis and indicators of oxidative stress. *Free Radic Biol Med* 32: 1090–1101, 2002.
58. Heinecke JW. Pathways for oxidation of low density lipoprotein by myeloperoxidase: tyrosyl radical, reactive aldehydes, hypochlorous acid and molecular chlorine. *Biofactors* 6: 145–155, 1997.
59. Heinecke JW, Rosen H, and Chait A. Iron and copper promote modification of low density lipoprotein by human arterial smooth muscle cells in culture. *J Clin Invest* 74: 1890–1894, 1984.
60. HOPE Study Group. Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. Heart Outcomes Prevention Evaluation Study Investigators. *Lancet* 355: 253–259, 2000.
61. Houstis N, Rosen ED, and Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* 440: 944–948, 2006.
62. Hu FB, Stampfer MJ, Solomon CG, Liu S, Willett WC, Speizer FE, Nathan DM, and Manson JE. The impact of diabetes mellitus on mortality from all causes and coronary heart disease in women: 20 years of follow-up. *Arch Intern Med* 161: 1717–1723, 2001.
63. Huggins TG, Staton MW, Dyer DG, Detorie NJ, Walla MD, Baynes JW, and Thorpe SR. *o*-Tyrosine and dityrosine concentrations in oxidized proteins and lens proteins with age. *Ann NY Acad Sci* 663: 436–437, 1992.
64. Huggins TG, Wells–Knecht MC, Detorie NA, Baynes JW, and Thorpe SR. Formation of *o*-tyrosine and dityrosine in proteins during radiolytic and metal-catalyzed oxidation. *J Biol Chem* 268: 12341–12347, 1993.
65. Hunt JV, Dean RT, and Wolff SP. Hydroxyl radical production and autoxidative glycosylation. Glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and ageing. *Biochem J* 256: 205–212, 1988.
66. Hunt JV, Smith CC, and Wolf SP. Oxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. *Diabetes* 39: 1420–1424, 1990.
67. Ido Y, Kilo C, and Williamson JR. Cytosolic NADH/NAD supply, free radicals and vascular dysfunction in early diabetes mellitus. *Diabetologia* 40: S115–S117, 1997.
68. Ihm S, Hyung J, Park S, and Ihm J. Effect of aminoguanidine on lipid-peroxidation in streptozotocin-induced diabetic rats. *Metabolism* 48: 1141–1145, 1999.
69. 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.
70. Ishii H, Daisuke K, and King GL. Protein kinase C activation and its role in the development of vascular complications in diabetes mellitus. *J Mol Med* 76: 21–31, 1998.
71. 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.
72. Jay D, Hitomi H, and Griendling KK. Oxidative stress and diabetic cardiovascular complications. *Free Radic Biol Med* 40: 183–192, 2006.
73. Kaur H and Halliwell B. Detection of hydroxyl radicals by aromatic hydroxylation. *Meth Enzymol* 233: 67–82, 1994.
74. Kawamura M, Heinecke JW, and Chait A. Pathophysiological concentrations of glucose promote oxidative modification of low density lipoprotein by a superoxide-dependent pathway. *J Clin Invest* 94: 771–778, 1994.
75. King GL, Ishii H, and Koya D. Diabetic vascular dysfunctions: a model of excessive activation of protein kinase C. K*idney Int Suppl* 60: S77–85, 1997.
76. Kita T, Nagano Y, Yokode M, Ishii K, Kume N, Ooshima A, Yoshida H, and Kawai C. Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. *Proc Natl Acad Sci USA* 84: 5928–5931, 1987.
77. Klebanoff SJ. Oxygen metabolism and the toxic properties of phagocytes. *Ann Intern Med* 93: 480–489, 1980.
78. Klebanoff SJ and Clark RA. *The Neutrophil: Function and Clinical Disorders.* Amsterdam: North Holland Biochemical Press, 1978, p. 447–451.
79. Klein R, Klien BE, and Moss SE. The Wisconsin Epidemiologic Study of Diabetic Retinopathy: a review. *Diabetes Metab Rev* 5: 559–570, 1989.
80. Lamharzi N, Renard CB, Kramer F, Pennathur S, Heinecke JW, Chait A, and Bornfeldt KE. Hyperlipidemia in concert with hyperglycemia stimulates the proliferation of macrophages in atherosclerotic lesions: potential role of glucose-oxidized LDL. *Diabetes* 53: 3217–3225, 2004.
81. Leeuwenburgh C, Hansen P, Shaish A, Holloszy JO, and Heinecke JW. Markers of protein oxidation by hydroxyl radical and reactive nitrogen species in tissues of aging rats. *Am J Physiol* 274: R453–461, 1998.
82. Leeuwenburgh C, Hansen PA, Holloszy JO, and Heinecke JW. Oxidized amino acids in the urine of aging rats: potential markers for assessing oxidative stress *in vivo*. *Am J Physiol* 276: R128–135, 1999.
83. Leeuwenburgh C, Hardy MM, Hazen SL, Wagner P, Oh-ishi S, Steinbrecher UP, and Heinecke JW. Reactive nitrogen intermediates promote low density lipoprotein oxidation in human atherosclerotic intima. *J Biol Chem* 272: 1433–1436, 1997.
84. Leeuwenburgh C, Rasmussen JE, Hsu FF, Mueller DM, Pennathur S, and Heinecke JW. Mass spectrometric quantification of markers for protein oxidation by tyrosyl radical, copper, and hydroxyl radical in low density lipoprotein isolated from human atherosclerotic plaques. *J Biol Chem* 272: 3520–3526, 1997.
85. Lorenzi M and Gerhardinger C. Early cellular and molecular changes induced by diabetes in the retina. *Diabetologia* 44: 791–804, 2001.
86. Maitra I, Serbinova E, Trischler H, and Packer L. a-Lipoic acid prevents buthionine sulfoximine-induced cataract formation in newborn rats. *Free Radical Biol Med* 18: 823–829, 1995.
87. Mashima R, Witting PK, and Stocker R. Oxidants and antioxidants in atherosclerosis. *Curr Opin Lipidol* 12: 411–418, 2001.

88. Mazzone T, Meyer PM, Feinstein SB, Davidson MH, Kondos GT, D'Agostino RB, Sr., Perez A, Provost JC, and Haffner SM. Effect of pioglitazone compared with glimepiride on carotid intima-media thickness in type 2 diabetes: a randomized trial. *JAMA* 296: 2572–2581, 2006.
89. McCance DR, Dyer DG, Dunn JA, Bailie KE, Thorpe SR, Baynes JW, and Lyons TJ. Maillard reaction products and their relation to complications in insulin-dependent diabetes mellitus. *J Clin Invest* 91: 2470–2478, 1993.
90. Meagher EA, Barry OP, Lawson JA, Rokach J, and FitzGerald GA. Effects of vitamin E on lipid peroxidation in healthy persons. *JAMA* 285: 1178–1182, 2001.
91. Mezzetti A, Cipollone F, and Cuccurullo F. Oxidative stress and cardiovascular complications of diabetes: isoprostanes as new markers on an old paradigm. *Cardiovas Res* 47: 475–488, 2000.
92. Miyata T, Fu MX, Kurokawa K, van Ypersele de Strihou C, Thorpe SR, and Baynes JW. Autoxidation products of both carbohydrates and lipids are increased in uremic plasma: is there oxidative stress in uremia? *Kidney Int* 54: 1290–1295, 1998.
93. Miyata T, Ueda Y, Yoshido A, Sugiyama S, Iida Y, Jadoul M, Maeda K, Kurokawa K, and van Ypersele de Strihou C. Clearance of pentosidine, an advanced glycation end-product, by different modalities of renal replacement therapy. *Kidney Int* 51: 880–887, 1997.
94. Miyata T, van Ypersele de Strihou C, Kurokawa K, and Baynes JW. Alterations in nonenzymatic biochemistry in uremia: origin and significance of "carbonyl stress" in long-term uremic complications. *Kidney Int* 55: 389–399, 1999.
95. Monnier VM. Transition metals redox: reviving an old plot for diabetic vascular disease. *J Clin Invest* 107: 799–801, 2001.
96. Morel DW, DiCorleto PE, and Chisolm GM. Endothelial and smooth muscle cells alter low density lipoprotein *in vitro* by free radical oxidation. *Arteriosclerosis* 4: 357–364, 1984.
97. Nagamatsu M, Nickander KK, Schmelzer JD, Ray A, Wittrock DA, Tritschler H, and Low PA. Lipoic acid improves nerve blood flow, reduces oxidative stress, and improves distal nerve conduction in experimental diabetic neuropathy. *Diabetes Care* 18: 1160–1167, 1995.
98. Nathan DM. Some answers, more controversy, from UKPDS. United Kingdom Prospective Diabetes Study. *Lancet* 352: 832–833, 1998.
99. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, and Zinman B. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 353: 2643–2653, 2005.
100. 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.
101. 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.
102. Oak JH and Cai H. Attenuation of angiotensin II signaling recouples eNOS and inhibits nonendothelial NOX activity in diabetic mice. *Diabetes* 56: 118–126, 2007.
103. Obrosova IG, Fathallah L, and Greene DA. Early changes in lipid peroxidation and antioxidative defense in diabetic rat retina: effect of DL-alpha-lipoic acid. *Eur J Pharmacol* 398: 139–146, 2000.
104. Onorato JM, Jenkins AJ, Thorpe SR, and Baynes JW. Pyridoxamine, an inhibitor of advanced glycation reactions, also inhibits advanced lipoxidation reactions. Mechanism of action of pyridoxamine. *J Biol Chem* 275: 21177–21184, 2000.
105. Packer L, Witt EH, and Tritschler HJ. Alpha-lipoic acid as a biological antioxidant. *Free Radical Biol Med* 19: 227–250, 1995.
106. Parthasarathy S, Young SG, Witztum JL, Pittman RC, and Steinberg D. Probucol inhibits oxidative modification of low density lipoprotein. *J Clin Invest* 77: 641–644, 1986.
107. Pennathur S, Bergt C, Shao B, Byun J, Kassim SY, Singh P, Green PS, McDonald TO, Brunzell J, Chait A, Oram JF, O'Brien K, Geary RL, and Heinecke JW. Human atherosclerotic intima and blood of patients with established coronary artery disease contain high density lipoprotein damaged by reactive nitrogen species. *J Biol Chem* 279: 42977–42983, 2004.
108. Pennathur S and Heinecke JW. Mechanisms of oxidative stress in diabetes: implications for the pathogenesis of vascular disease and antioxidant therapy. *Front Biosci* 9: 565–574, 2004.
109. Pennathur S, Ido Y, Heller JI, Byun J, Danda R, Pergola P, Williamson JR, and Heinecke JW. Reactive carbonyls and polyunsaturated fatty acids produce a hydroxyl radical-like species: a potential pathway for oxidative damage of retinal proteins in diabetes. *J Biol Chem* 280: 22706–22714, 2005.
110. Pennathur S, Jackson–Lewis V, Przedborski S, and Heinecke JW. Mass spectrometric quantification of 3-nitrotyrosine, ortho-tyrosine, and o,o'-dityrosine in brain tissue of 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine-treated mice, a model of oxidative stress in Parkinson's disease. *J Biol Chem* 274: 34621–34628, 1999.
111. Pennathur S, Wagner JD, Leeuwenburgh C, Litwak KN, and Heinecke JW. A hydroxyl radical-like species oxidizes cynomolgus monkey artery wall proteins in early diabetic vascular disease. *J Clin Invest* 107: 853–860, 2001.
112. Pfeiffer A and Schatz H. Diabetic microvascular complications and growth factors. *Exp Clin Endocrinol Diabetes* 103: 7–14, 1995.
113. Pilz S, Scharnagl H, Tiran B, Seelhorst U, Wellnitz B, Boehm BO, Schaefer JR, and Marz W. Free fatty acids are independently associated with all-cause and cardiovascular mortality in subjects with coronary artery disease. *J Clin Endocrinol Metab* 91: 2542–2547, 2006.
114. Requena JR, Ahmed MU, Fountain CW, Degenhardt TP, Reddy S, Perez C, Lyons TJ, Jenkins AJ, Baynes JW, and Thorpe SR. Carboxymethylethanolamine, a biomarker of phospholipid modification during the maillard reaction *in vivo*. *J Biol Chem* 272: 17473–17479, 1997.
115. Requena JR, Fu MX, Ahmed MU, Jenkins AJ, Lyons TJ, Baynes JW, and Thorpe SR. Quantification of malondialdehyde and 4-hydroxynonenal adducts to lysine residues in native and oxidized human low-density lipoprotein. *Biochem J* 322 (Pt 1): 317–325, 1997.
116. Requena JR, Fu MX, Ahmed MU, Jenkins AJ, Lyons TJ, and Thorpe SR. Lipoxidation products as biomarkers of oxidative damage to proteins during lipid peroxidation reactions. *Nephrol Dial Transplant* 11 suppl 5: 48–53, 1996.
117. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 340: 115–126, 1999.
118. Salvemini D, Wang Z, Zwier JL, Samouilov A, Macarthur H, Misko T, Currie MG, Cuzzocrea S, Sikorski JA, and Riley DP. A nonpeptidyl mimic of superoxide dismutase with therapeutic activity in rats. *Science* 286: 304–306, 1999.
119. Savenkova ML, Mueller DM, and Heinecke JW. Tyrosyl radical generated by myeloperoxidase is a physiological catalyst for the initiation of lipid peroxidation in low density lipoprotein. *J Biol Chem* 269: 20394–20400, 1994.
120. Schmidt AM, Hori O, Cao R, Yan SD, Brett J, Wautier JL, Ogawa S, Kuwabara K, Matsumoto M, and Stern D. RAGE: a novel cellular receptor for advanced glycation end products. *Diabetes* 45 suppl 3: S77–80, 1996.
121. Sell DR, Lapolla A, Odetti P, Fogarty J, and Monnier VM. Pentosidine formation in skin correlates with the severity of complications in individuals with long -standing IDDM. *Diabetes* 41: 1286–1292, 1992.
122. Semenkovich CF and Heinecke JW. The mystery of diabetes and atherosclerosis: time for a new plot. *Diabetes* 46: 327–334, 1997.
123. Shao B, Oda MN, Oram JF, and Heinecke JW. Myeloperoxidase: an inflammatory enzyme for generating dysfunctional high density lipoprotein. *Curr Opin Cardiol* 21: 322–328, 2006.
124. Sharma K and Ziyadeh FN. Biochemical events and cytokine interactions linking glucose metabolism to the development of diabetic nephropathy. *Sem Nephrol* 17: 80–92, 1997.
125. Shishehbor MH, Aviles RJ, Brennan ML, Fu X, Goormastic M, Pearce GL, Gokce N, Keaney JF Jr, Penn MS, Sprecher DL, Vita JA, and Hazen SL. Association of nitrotyrosine levels with car-

diovascular disease and modulation by statin therapy. *JAMA* 289: 1675–1680, 2003.
126. Shishehbor MH, Brennan ML, Aviles RJ, Fu X, Penn MS, Sprecher DL, and Hazen SL. Statins promote potent systemic antioxidant effects through specific inflammatory pathways. *Circulation* 108: 426–431, 2003.
127. Shishehbor MH and Hazen SL. Inflammatory and oxidative markers in atherosclerosis: relationship to outcome. *Curr Atheroscler Rep* 6: 243–250, 2004.
128. Sonta T, Inoguchi T, Tsubouchi H, Sekiguchi N, Kobayashi K, Matsumoto S, Utsumi H, and Nawata H. Evidence for contribution of vascular NAD(P)H oxidase to increased oxidative stress in animal models of diabetes and obesity. *Free Radic Biol Med* 37: 115–123, 2004.
129. Steinberg D. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nat Med* 8: 1211–1217, 2002.
130. Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, and Steinberg D. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc Natl Acad Sci USA* 81: 3883–3887, 1984.
131. Stern DM, Yan SD, Yan SF, and Schmidt AM. Receptor for advanced glycation endproducts (RAGE) and the complications of diabetes. *Ageing Res Rev* 1: 1–15, 2002.
132. Stitt A, Gardiner TA, Alderson NL, Canning P, Frizzell N, Duffy N, Boyle C, Januszewski AS, Chachich M, Baynes JW, and Thorpe SR. The AGE inhibitor pyridoxamine inhibits development of retinopathy in experimental diabetes. *Diabetes* 51: 2826–2832, 2002.
133. UKPDS Research Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group [see comments] [published erratum appears in Lancet 1998 Nov 7;352:1557]. *Lancet* 352: 854–865, 1998.
134. UKPDS Research 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). UK Prospective Diabetes Study (UKPDS) Group [see comments]. *Lancet* 352: 837–853, 1998.
135. Van Gaal LF, Mertens IL, and De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature* 444: 875–880, 2006.
136. Vasquez–Vivar J, Kalyanaraman B, Martasek P, Hogg N, Masters BS, Karoui H, Tordo P, and Pritchard KA, Jr. Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. *Proc Natl Acad Sci USA* 95: 9220–9225, 1998.
137. Voziyan PA, Metz TO, Baynes JW, and Hudson BG. A post-Amadori inhibitor pyridoxamine also inhibits chemical modification of proteins by scavenging carbonyl intermediates of carbohydrate and lipid degradation. *J Biol Chem* 277: 3397–3403, 2002.
138. 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.
139. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, Charo I, Leibel RL, and Ferrante AW, Jr. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *J Clin Invest* 116: 115–124, 2006.
140. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, and Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112: 1796–1808, 2003.
141. Wells–Knecht MC, Lyons TJ, McCance DR, Thorpe SR, and Baynes JW. Age-dependent increase in ortho-tyrosine and methionine sulfoxide in human skin collagen is not accelerated in diabetes. Evidence against a generalized increase in oxidative stress in diabetes. *J Clin Invest* 100: 839–846, 1997.
142. Wild S, Roglic G, Green A, Sicree R, and King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27: 1047–1053, 2004.
143. Williamson JR, Chang K, Frangos M, Hasan KS, Ido Y, Kawamura T, Nyengaard JR, van den Enden M, Kilo C, and Tilton RG. Hyperglycemic pseudohypoxia and diabetic complications. *Diabetes* 42: 801–813, 1993.
144. Witztum JL and Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest* 88: 1785–1792, 1991.
145. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, and Kahn BB. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 436: 356–362, 2005.
146. Yla–Herttuala S, Palinski W, Butler SW, Picard S, Steinberg D, and Witztum JL. Rabbit and human atherosclerotic lesions contain IgG that recognizes epitopes of oxidized LDL. *Arterioscler Thromb* 14: 32–40, 1994.
147. Yla–Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, Butler S, Witztum JL, and Steinberg D. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J Clin Invest* 84: 1086–1095, 1989.
148. Yusuf S, Dagenais G, Pogue J, Bosch J, and Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 342: 154–160, 2000.
149. Zhang SX, Sima J, Shao C, Fant J, Chen Y, Rohrer B, Gao G, and Ma JX. Plasminogen kringle 5 reduces vascular leakage in the retina in rat models of oxygen-induced retinopathy and diabetes. *Diabetologia* 47: 124–131, 2004.
150. Zheng L, Nukuna B, Brennan ML, Sun M, Goormastic M, Settle M, Schmitt D, Fu X, Thomson L, Fox PL, Ischiropoulos H, Smith JD, Kinter M, and Hazen SL. Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J Clin Invest* 114: 529–541, 2004.
151. Ziegler D, Hanefeld M, Ruhnau KJ, Hasche H, Lobisch M, Schutte K, Kerum G, and Malessa R. 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 Neuropathy. *Diabetes Care* 22: 1296–1301, 1999.
152. Ziegler D, Reljanovic M, Mehnert H, and Gries FA**.** Alpha-lipoic acid in the treatment of diabetic polyneuropathy in Germany: current evidence from clinical trials. *Exp Clin Endocrinol Diabetes* 107: 421–430, 1999.
153. Ziegler D, Schatz H, Conrad F, Gries FA, Ulrich H, and Reichel G. 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). Deutsche Kardiale Autonome Neuropathie. *Diabetes Care* 20: 369–373, 1997.

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2. Sushil K. Jain, Gunjan Kahlon, Lester Morehead, Richa Dhawan, Benjamin Lieblong, Tommie Stapleton, Gloria Caldito, Robert Hoeldtke, Steven N. Levine, Pat Farrington Bass. 2012. Effect of chromium dinicocysteinate supplementation on circulating levels of insulin, TNF-#, oxidative stress, and insulin resistance in type 2 diabetic subjects: Randomized, double-blind, placebo-controlled study. *Molecular Nutrition & Food Research* **56**:8, 1333-1341. [CrossRef]
3. K. Saito, K. Odashiro, T. Maruyama, K. Akashi, S. Mawatari, T. Fujino. 2012. Improvement of diabetic or obese patients' erythrocyte deformability by the program of the brain-oriented obesity control system (BOOCS). *The Journal of Physiological Sciences* . [CrossRef]
4. Serena Del Turco, Giuseppina Basta. 2012. An update on advanced glycation endproducts and atherosclerosis. *BioFactors* **38**:4, 266-274. [CrossRef]
5. Nadina Stadler, Sylvia Heeneman, Stefan Vöö, Naomi Stanley, Gregory I. Giles, Bevan P. Gang, Kevin D. Croft, Trevor A. Mori, Vladimir Vacata, Mat J.A.P. Daemen, Johannes Waltenberger, Michael J. Davies. 2012. Reduced metal ion concentrations in atherosclerotic plaques from subjects with Type 2 diabetes mellitus. *Atherosclerosis* **222**:2, 512-518. [CrossRef]
6. Michael J. DaviesOxidative Damage to Proteins . [CrossRef]
7. Yi-Chen Liao, Ying-Ho Lee, Lea-Yea Chuang, Jinn-Yuh Guh, Ming-Der Shi, Jau-Shyang Huang. 2012. Advanced Glycation End Products-Mediated Hypertrophy Is Negatively Regulated by Tetrahydrobiopterin in Renal Tubular Cells. *Molecular and Cellular Endocrinology* . [CrossRef]
8. Serena Del Turco, Giuseppina Basta. 2012. An update on advanced glycation endproducts and atherosclerosis. *BioFactors* n/a. [CrossRef]
9. Nhat-Tu Le, James P. Corsetti, Janet L. Dehoff-Sparks, Charles E. Sparks, Keigi Fujiwara, Jun-ichi Abe. 2012. Reactive Oxygen Species, SUMOylation, and Endothelial Inflammation. *International Journal of Inflammation* **2012**, 1-13. [CrossRef]
10. Ming-Hui Zou, Hongliang Li, Chaoyong He, Mingkai Lin, Timothy J. Lyons, Zhonglin Xie. 2011. Tyrosine Nitration of Prostacyclin Synthase Is Associated with Enhanced Retinal Cell Apoptosis in Diabetes. *The American Journal of Pathology* **179**:6, 2835-2844. [CrossRef]
11. Zhigang Wang, Xiaobing Dou, Dongfang Gu, Chen Shen, Tong Yao, Van Nguyen, Carol Braunschweig, Zhenyuan Song. 2011. 4-Hydroxynonenal differentially regulates adiponectin gene expression and secretion via activating PPAR# and accelerating ubiquitin–proteasome degradation. *Molecular and Cellular Endocrinology* . [CrossRef]
12. Husam M. Abu-Soud, Dhiman Maitra, Jaeman Byun, Carlos Eduardo A. Souza, Jashoman Banerjee, Ghassan M. Saed, Michael P. Diamond, Peter R. Andreana, Subramaniam Pennathur. 2011. The reaction of HOCl and cyanocobalamin: Corrin destruction and the liberation of cyanogen chloride. *Free Radical Biology and Medicine* . [CrossRef]
13. Meiling Yan, Jawahar L. Mehta, Weifang Zhang, Changping Hu. 2011. LOX-1, Oxidative Stress and Inflammation: A Novel Mechanism for Diabetic Cardiovascular Complications. *Cardiovascular Drugs and Therapy* . [CrossRef]
14. Paige S. Katz, Aaron J. Trask, Flavia M. Souza-Smith, Kirk R. Hutchinson, Maarten L. Galantowicz, Kevin C. Lord, James A. Stewart, Mary J. Cismowski, Kurt J. Varner, Pamela A. Lucchesi. 2011. Coronary arterioles in type 2 diabetic (db/db) mice undergo a distinct pattern of remodeling associated with decreased vessel stiffness. *Basic Research in Cardiology* . [CrossRef]
15. Lu Luo, De-Zai Dai, Yu-Si Cheng, Qi Zhang, Wen-Jun Yuan, Yin Dai. 2011. Sildenafil improves diabetic vascular activity through suppressing endothelin receptor A, iNOS and NADPH oxidase which is comparable with the endothelin receptor antagonist CPU0213 in STZ-injected rats. *Journal of Pharmacy and Pharmacology* **63**:7, 943-951. [CrossRef]
16. Jung-Mi Yun, Ishwarlal Jialal, Sridevi Devaraj. 2011. Epigenetic regulation of high glucose-induced proinflammatory cytokine production in monocytes by curcumin. *The Journal of Nutritional Biochemistry* **22**:5, 450-458. [CrossRef]
17. Yupin Li, Takeki Hamasaki, Noboru Nakamichi, Taichi Kashiwagi, Takaaki Komatsu, Jun Ye, Kiichiro Teruya, Masumi Abe, Hanxu Yan, Tomoya Kinjo, Shigeru Kabayama, Munenori Kawamura, Sanetaka Shirahata. 2011. Suppressive effects of electrolyzed reduced water on alloxan-induced apoptosis and type 1 diabetes mellitus. *Cytotechnology* **63**:2, 119-131. [CrossRef]
18. Sandra Fernanda Nunes, Isabel Vitoria Figueiredo, João Santos Pereira, Paulo João Soares, Maria Margarida Caramona, Brian Callingham. 2010. Changes in the activities of semicarbazide-sensitive amine oxidase in inferior mesenteric artery segments and in serum of patients with type 2 diabetes. *Acta Diabetologica* **47**:2, 179-182. [CrossRef]
19. Merja K. Kataja-Tuomola, Jukka P. Kontto, Satu Männistö, Demetrius Albanes, Jarmo R. Virtamo. 2010. Effect of alpha-tocopherol and beta-carotene supplementation on macrovascular complications and total mortality from diabetes: Results of the ATBC Study. *Annals of Medicine* **42**:3, 178-186. [CrossRef]
20. Agnès Matheson, Mark D. P. Willcox, Judith Flanagan, Bradley J. Walsh. 2010. Urinary biomarkers involved in type 2 diabetes: a review. *Diabetes/Metabolism Research and Reviews* **26**:3, 150-171. [CrossRef]

21. Giancarlo Aldini , Giulio Vistoli , Luca Regazzoni , Maria Carmela Benfatto , Ilaria Bettinelli , Marina Carini . 2010. Edaravone Inhibits Protein Carbonylation by a Direct Carbonyl-Scavenging Mechanism: Focus on Reactivity, Selectivity, and Reaction Mechanisms. *Antioxidants & Redox Signaling* **12**:3, 381-392. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
22. Mark P. Mattson. 2009. Roles of the lipid peroxidation product 4-hydroxynonenal in obesity, the metabolic syndrome, and associated vascular and neurodegenerative disorders. *Experimental Gerontology* **44**:10, 625-633. [CrossRef]
23. Kyoung Ah Kang, Jin Sook Kim, Rui Zhang, Mei Jing Piao, Dong Ok Ko, Zhi Hong Wang, Young Jun Heo, Doek Bae Park, Young Hee Maeng, Jin Won Hyun. 2009. Effect of KIOM-79 Against Mitochondrial Damage Induced by Streptozotocin in Pancreatic #-Cells. *Journal of Toxicology and Environmental Health, Part A* **72**:20, 1201-1208. [CrossRef]
24. Rodica Pop-Busui, Elif Oral, David Raffel, Jaeman Byun, Valida Bajirovic, Anuradha Vivekanandan-Giri, Aaron Kellogg, Subramaniam Pennathur, Martin J. Stevens. 2009. Impact of rosiglitazone and glyburide on nitrosative stress and myocardial blood flow regulation in type 2 diabetes mellitus. *Metabolism* **58**:7, 989-994. [CrossRef]
25. Giulio Vistoli, Marica Orioli, Alessandro Pedretti, Luca Regazzoni, Renato Canevotti, Gianpaolo Negrisoli, Marina Carini, Giancarlo Aldini. 2009. Design, Synthesis, and Evaluation of Carnosine Derivatives as Selective and Efficient Sequestering Agents of Cytotoxic Reactive Carbonyl Species. *ChemMedChem* **4**:6, 967-975. [CrossRef]
26. Yehudi Self-Medlin, Jungsoo Byun, Robert F. Jacob, Yoshiko Mizuno, R. Preston Mason. 2009. Glucose promotes membrane cholesterol crystalline domain formation by lipid peroxidation. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1788**:6, 1398-1403. [CrossRef]
27. Nica M. Borradaile, J. Geoffrey Pickering. 2009. Nicotinamide phosphoribosyltransferase imparts human endothelial cells with extended replicative lifespan and enhanced angiogenic capacity in a high glucose environment. *Aging Cell* **8**:2, 100-112. [CrossRef]
28. Sushil K. Jain , Justin Rains , Jennifer Croad , Bryon Larson , Kimberly Jones . 2009. Curcumin Supplementation Lowers TNF-#, IL-6, IL-8, and MCP-1 Secretion in High Glucose-Treated Cultured Monocytes and Blood Levels of TNF-#, IL-6, MCP-1, Glucose, and Glycosylated Hemoglobin in Diabetic Rats. *Antioxidants & Redox Signaling* **11**:2, 241-249. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
29. H SHERTZER, S SCHNEIDER, E KENDIG, D CLEGG, D DALESSIO, E JOHANSSON, M GENTER. 2009. Tetrahydroindenoindole inhibits the progression of diabetes in mice. *Chemico-Biological Interactions* **177**:1, 71-80. [CrossRef]
30. John W. Baynes, David B. Murray. 2009. The Metal Chelators, Trientine and Citrate, Inhibit the Development of Cardiac Pathology in the Zucker Diabetic Rat. *Experimental Diabetes Research* **2009**, 1-6. [CrossRef]
31. Valerije Vrc#ek, Hrvoje Mes#tric#. 2009. Chlorination of ammonia and aliphatic amines by Cl 2 : DFT study of medium and substituent effects. *Journal of Physical Organic Chemistry* **22**:1, 59-68. [CrossRef]
32. S. Kopprasch, J. Pietzsch, I. Ansurudeen, J. Graessler, A. W Krug, M. Ehrhart-Bornstein, S. R Bornstein. 2009. Prediabetic and diabetic in vivo modification of circulating low-density lipoprotein attenuates its stimulatory effect on adrenal aldosterone and cortisol secretion. *Journal of Endocrinology* **200**:1, 45-52. [CrossRef]
33. Anuradha Vivekanadan-Giri, Jeffrey H. Wang, Jaeman Byun, Subramaniam Pennathur. 2008. Mass spectrometric quantification of amino acid oxidation products identifies oxidative mechanisms of diabetic end-organ damage. *Reviews in Endocrine and Metabolic Disorders* **9**:4, 275-287. [CrossRef]
34. Muyao Li, Jen-Fu Chiu, Jessica Gagne, Naomi K. Fukagawa. 2008. Age-related differences in insulin-like growth factor-1 receptor signaling regulates Akt/FOXO3a and ERK/Fos pathways in vascular smooth muscle cells. *Journal of Cellular Physiology* **217**:2, 377-387. [CrossRef]
35. Antonio Ceriello, Katherine Esposito, Ludovica Piconi, Michael Ihnat, Jessica Thorpe, Roberto Testa, Anna Rita Bonfigli, Dario Giugliano. 2008. Glucose “peak” and glucose “spike”: Impact on endothelial function and oxidative stress. *Diabetes Research and Clinical Practice* **82**:2, 262-267. [CrossRef]
36. Robert L Wilensky, Yi Shi, Emile R Mohler, Damir Hamamdzic, Mark E Burgert, Jun Li, Anthony Postle, Robert S Fenning, James G Bollinger, Bryan E Hoffman, Daniel J Pelchovitz, Jisheng Yang, Rosanna C Mirabile, Christine L Webb, LeFeng Zhang, Ping Zhang, Michael H Gelb, Max C Walker, Andrew Zalewski, Colin H Macphee. 2008. Inhibition of lipoprotein-associated phospholipase A2 reduces complex coronary atherosclerotic plaque development. *Nature Medicine* **14**:10, 1059-1066. [CrossRef]
37. Philippe Rondeau, Nihar Ranjan Singh, Henri Caillens, Frank Tallet, Emmanuel Bourdon. 2008. Oxidative stresses induced by glycoxidized human or bovine serum albumin on human monocytes. *Free Radical Biology and Medicine* **45**:6, 799-812. [CrossRef]
38. Michael J. Davies , Clare L. Hawkins , David I. Pattison , Martin D. Rees . 2008. Mammalian Heme Peroxidases: From Molecular Mechanisms to Health Implications. *Antioxidants & Redox Signaling* **10**:7, 1199-1234. [Abstract] [Full Text PDF] [Full Text PDF with Links]
39. Weihua Liu, Peiqinq Liu, Sha Tao, Yanhui Deng, Xuejuan Li, Tian Lan, Xiaoyan Zhang, Fenfen Guo, Wenge Huang, Fengying Chen, Heqing Huang, Shu-Feng Zhou. 2008. Berberine inhibits aldose reductase and oxidative stress in rat mesangial cells cultured under high glucose. *Archives of Biochemistry and Biophysics* **475**:2, 128-134. [CrossRef]
40. Theodore Mazzone, Alan Chait, Jorge Plutzky. 2008. Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. *The Lancet* **371**:9626, 1800-1809. [CrossRef]

41. Hamid Mirzaei, Beatriz Baena, Coral Barbas, Fred Regnier. 2008. Identification of oxidized proteins in rat plasma using avidin chromatography and tandem mass spectrometry. *PROTEOMICS* **8**:7, 1516-1527. [CrossRef]
42. Prasenjit Manna, Mahua Sinha, Parames C. Sil. 2008. Protection of arsenic-induced testicular oxidative stress by arjunolic acid. *Redox Report* **13**:2, 67-77. [CrossRef]
43. M.E.H. Mazumder, S. Rahman. 2008. Pharmacological Evaluation of Bangladeshi Medicinal Plants for Antioxidant Activity. *Pharmaceutical Biology* **46**:10-11, 704-709. [CrossRef]
44. Melvin R. Hayden , James R. Sowers . 2007. Redox Imbalance in Diabetes. *Antioxidants & Redox Signaling* **9**:7, 865-867. [Citation] [Full Text PDF] [Full Text PDF with Links]
45. Ahmet Cumaog#lu, ÇEmal Cevik, Lucia Rackova, Nuray Ari, ÇImen Karasu. 2007. Effects of antioxidant stobadine on protein carbonylation, advanced oxidation protein products and reductive capacity of liver in streptozotocin-diabetic rats: Role of oxidative/nitrosative stress. *BioFactors* **30**:3, 171-178. [CrossRef]