Oxidative Stress and Disease: An Updated Review

Amira A.M. Adly
Diabetes Unit, Department of Pediatrics, Ain Shams University, Cairo, Egypt

INTRODUCTION

Definition

Oxidative stress is the presence of active oxygen species (ROS) in excess of the available antioxidant buffering capacity (Fig. 1). These reactive oxygen species can damage proteins, lipids and DNA, altering the organism’s structure and function (Roberts and Hubel, 2004). Sies and Cadenas (1985) introduced the term from the book.

Definition of Reactive Species

Most stable molecular species have the electrons in their outer orbital, arranged in pairs. Each electron of this pair has an opposite spin, which is important to stabilize the molecules. A free radical is a molecule with one or more unpaired electrons in its outer orbital, which makes this specie very unstable and tending to react with other molecules to pair this electron and thereby generate more stable specie (Guteers et al., 2002).

Types of Reactive Species

The highly reactive molecules include Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) as shown in Fig. 2 (Martín et al., 2003). Both types were listed in Table 1 and 2.

Fig. 1: Imbalance between oxidant and antioxidant (Garrido et al., 2004)
Table 1: Reactive Oxygen Species (ROS)

<table>
<thead>
<tr>
<th>Radicals</th>
<th>Non-radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide: O₂⁻</td>
<td>Hydrogen peroxide: H₂O₂</td>
</tr>
<tr>
<td>Hydroxyl: OH⁻</td>
<td>Hypochlorous acid: HOCL</td>
</tr>
<tr>
<td>Peroxyl: RO₂⁻</td>
<td>Hypobromous acid: HOBr</td>
</tr>
<tr>
<td>Alkoxy: RO⁻</td>
<td>Ozone: O₃</td>
</tr>
<tr>
<td>Hydroperoxyl: HO₂⁻</td>
<td>Singlet oxygen: Δg</td>
</tr>
</tbody>
</table>

Source: Caimi et al. (2004)

Table 2: Reactive Nitrogen Species (RNS)

<table>
<thead>
<tr>
<th>Radicals</th>
<th>Non-radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitric oxide: NO⁻</td>
<td>Nitrogen dioxide: NO₂</td>
</tr>
<tr>
<td>Nitrous acid: NH₂O</td>
<td>Nitrosyl cation: NO</td>
</tr>
<tr>
<td></td>
<td>Nitrosyl anion: NO NO⁻</td>
</tr>
<tr>
<td></td>
<td>Dinitrogen tetroxide: N₂O₄</td>
</tr>
<tr>
<td></td>
<td>Dinitrogen trioxide: N₂O₃</td>
</tr>
<tr>
<td></td>
<td>Peroxynitrite: ONOO⁻</td>
</tr>
<tr>
<td></td>
<td>Peroxynitrous acid: ONOOH</td>
</tr>
<tr>
<td></td>
<td>Alkylperoxynitrites: ROONO</td>
</tr>
</tbody>
</table>

Source: Caimi et al. (2004)

Of these reactive molecules (•O₂⁻, •NO, •ONOO⁻) are the most widely studied species and play important roles in the diabetic cardiovascular complications. Superoxide (•O₂⁻) is produced by one electron reduction of oxygen by different oxidases including dihydro nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidase, cyclooxygenase as well as by the mitochondrial electron transport chain during the course of normal oxidative phosphorylation which is essential for generating ATP (Evans et al., 2003) (Fig. 3).

Sources of Free Radicals

The human body is continuously exposed to potentially harmful oxidative stresses during the course of life time. These may arise from exogenous as well as endogenous sources (Fig. 4) (Ray et al., 2001).
Fig. 3: Oxidative Stress pathways (www.sapphirebioscience.com 2008)

Fig. 4: Common sources of oxidative stress (Cadenas and Davies, 2000)
• **Endogenous sources of ROS**: The main source of ROS in vivo is aerobic respiration. ROS are also produced by peroxisomal β-oxidation of fatty acids, microsomal cytochrome P450 metabolism of xenobiotic compounds, stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism and tissue specific enzymes (Nicholls and Budd, 2000)

• **Exogenous sources of ROS**: Excessive levels of free radicals are produced from: pollution exposure; toxin exposure, including exotoxins such as heavy metals like mercury, lead and cadmium. Other exotoxins include: anticancer drugs, anesthetics and analgesics. Toxin exposure also includes endotoxins such as those produced from bacteria, yeast, viruses and parasites; trauma; radiation; electromagnetic fields; alcohol; cigarette smoke; medications; stress; allergens; cold; excessive exercise; dietary factors such as excess sugar, saturated fat and fried oils; malnutrition and various disease states (Cadenas and Davies, 2000)

**Physiological Functions of Free Radicals**

Free radicals, especially those centered on oxygen have well defined functions and play a pivotal role in many physiologic reactions, as catalytic oxidation of some endogenous compounds and xenobiotics. Furthermore, they are very important participants in the regulation of smooth muscle tone and bacterial function of phagocytes (Droge, 2002).

**Biological Actions of Free Radicals**

Oxidative stress can cause damage to all molecular targets; DNA, proteins and lipids (Fig. 5), often, it is not clear which is the first point of attack, since injury mechanisms overlap widely. The primary cellular target of OS can vary; DNA is an important early target of damage (Guteens et al., 2002).

**Oxidative Lipid Damage**

Although lipid peroxidation (LP) affects many cellular components, the primary action sites involve membrane associated polyunsaturated fatty acids (PUFA). Peroxidation of membrane associated fatty acids and cholesterol will alter cell membrane fluidity and

![Diagram of biological actions of free radicals](https://www.biozentrum.uni-freiburg.de/Pharmazie/index.html)

**Fig. 5**: Biological actions of free radicals (Guteens et al., 2002)
permeability characteristics and may eventually induce widespread membrane damage. Lipid peroxides arising as a consequence of tissue damage can propagate further LP locally and at sites distant to areas of initial damage. The lipid peroxidation chain can lead to an elevated steady state concentration of lipid peroxides at the expense of oxygen and unsaturated lipids (Betteridge, 2000).

Further decomposition of peroxidized lipids yields a wide variety of end products, including (MDA) which is used as a marker of free radical mediated reactions. LP can damage membrane proteins and lipids. Hydroperoxides are stable products formed during the peroxidation of unsaturated lipids such as fatty acids and cholesterol (De Andrade Junior et al., 2005).

Oxidative DNA Damage

Oxygen derived radicals may directly attack DNA, either the sugar, phosphate or purine and pyrimidine bases. On the other hand, oxidative DNA damage may be indirect through the rise of intracellular \( \text{Cu}^{+} \) ions. Free radical-mediated reactions can cause structural alterations in DNA (e.g., nicking, base-pair mutations, rearrangement, deletions, insertions and sequence amplification). Degradation of the bases will produce numerous products, including 8-OH-Gua, hydroxymethylurea, urea, thymine glycol, thymine and adenine ring opened and saturated products (Rowe et al., 2008).

Oxidative Protein Damage

Free radical injury results in inactivation and denaturation of essential proteins, the most at risk proteins are those with amino acids containing sulphur (methionine and cysteine), such as: some enzymes and membrane ion transporters. The radical abstracts a proton and thereby oxidizes the sulphydryl moiety. The enzymes at risk include alpha-1 antiprotease, calmodulin, calcium ATPase, glucose-6-phosphate dehydrogenase and glyceraldehyde-3phosphate dehydrogenase (Calissol et al., 2000).

Oxidative Stress and Disease

Oxidative stress can cause tissue injury or even cell death which can occur essentially by two mechanisms, necrosis and apoptosis, Fig. 6 (Gueuteens et al., 2002).

There is growing evidence of the involvement of free radical in disease processes, studies show that oxidized LDL is taken up by macrophages and foam cells more readily than is normal LDL. Free radicals produce structural damage to every tissue in the body and contribute to disease generation via activation of gene regulatory proteins (Van Wijk et al., 2008).

Oxidative stress plays a role in inflammation, accelerates aging and contributes in variety of degenerative conditions as in Fig. 7 e.g., cardiovascular diseases, atherosclerosis, cancer, cataract, central nervous system disorders, Parkinson's disease, Alzheimer's disease, inflammatory bowel disease, rheumatoid arthritis, diabetes, respiratory diseases, autoimmune diseases, liver diseases, kidney diseases, skin conditions and AIDS (Galli et al., 2005).

Cellular Defenses Against Oxidative Stress

Cells manifest potent antioxidant defenses against ROS, including detoxifying enzymes and exogenous free radical scavengers (vitamins). The major enzymes that convert ROS to less reactive molecules are superoxide dismutase, catalase and glutathione peroxidase (Zhu et al., 2008).
Fig. 6: Mechanisms of oxidative stress-induced cell damage (Agarwal et al., 2005)

In healthy individuals, antioxidants form the body's primary defense against ROS. They scavenge ROS before they cause damage to various biological molecules and prevent oxidative damage from spreading, by interrupting the free radical chain reaction. Antioxidants donate an electron to the free radical (Wang et al., 2008). Fruits and vegetables are the principal dietary contributors of antioxidants, being particularly rich in vitamins (A, C, E), oligoelements and polyphenols (Fig. 8) (Blokhina et al., 2003).

**Oxidative Stress and Diabetes**

Cardiovascular complications, characterized by endothelial dysfunction and accelerated atherosclerosis, are the leading cause of morbidity and mortality associated with diabetes.
(Salem et al., 2009). There is growing evidence that excess generation of highly reactive free radicals, largely due to hyperglycemia causes OS, which further exacerbates the development of diabetes complications. Overproduction and/or insufficient removal of these free radicals result in vascular dysfunction, damage to cellular proteins, membrane lipids and nucleic acids. Stabilizing glucose levels near normal levels is of utmost important (Johansen et al., 2005).
Pathological Consequences of OS in Diabetes

Reactive oxygen species are generated under physiological conditions and are involved to some extent as signaling molecules and defense mechanisms as seen in phagocytosis, neutrophil function and shear-stress induced vasorelaxation, excessive generation of ROS has pathological consequences including damage to proteins, lipids and DNA (Boullier et al., 2001).

Type 1 Diabetes Mellitus (DM1) is the effect of T cell dependant autoimmune destruction of insulin producing beta cells in the pancreatic islets. T cells are activated in response to islet dominant autoantigens, the result being the development of type 1 diabetes mellitus. Apoptosis is a highly regulated form of cell death defined by distinct morphological and biochemical features. It is a coordinated series of events for the programmed cell death and plays an important role in the maintenance of tissue homeostasis, embryonic development and in the control of immune responses in humans.

Fas (Apo-1/CD95) is a 45-KDa surface receptor belonging to the nerve growth factor superfamily, which on binding by Fas ligand (FasL) induces translocation of phosphatidyserine from the inner to the outer leaflet of the cellular membrane and directly transduces the signal for programmed cell death. Defective regulation of leukocyte apoptosis may be a factor which contributes to the pathogenic mechanism of autoimmune disease. It was found that immunological, inflammatory and metabolic signals cause β-cell apoptosis and that these signals converge toward a common β-cell death signaling pathway.

An Egyptian study on forty type 1 diabetic patients revealed a significant increase in CD95 percentage expression in DM1 in comparison to controls and they explain that by that the findings of immunological, inflammatory and metabolic signals leading to β-cell apoptosis were increased in diabetic patients and they proposed that these signals converge toward a common β-cell death signaling pathway (Sherif et al., 2005).

The increased generation of free radicals in the hyperglycemic state may lead to the production of advanced glycation end products and the peroxidation reaction in lipids and protein. Moreover DNA is also vulnerable to the action of free radicals. Thus, chronic hyperglycemia at the onset of diabetes may be associated with increased genotoxicity and apoptosis, thus has an impact on DNA repair machinery.

For example:

- $\text{H}_2\text{O}_2$ mediates apoptosis and pathological angiogenesis (Taniyama and Griendling, 2003)
- ROS can stimulate oxidation of Low-Density Lipoprotein (LDL) and αx-LDL, which is not recognized by the LDL receptor, can be taken up by scavenger receptors in macrophages leading to foam cell formation and atherosclerotic plaques (Boullier et al., 2001)
- ROS interfere with the transcription of insulin promoter gene and cause protein glycation and cross-linking, pancreatic fibrosis and lipid peroxidation (Cerillo and Motz, 2004). Superoxide ($\text{O}_2^-\text{)}$ can activate several damaging pathways in diabetes including accelerated formation of advanced glycation end products (AGE), polyol pathway, hexosamine pathway and PKC, all of which have been proven to be involved in micro- and macrovascular complications (Aronson, 2008)
- Furthermore, $\text{O}_2^-\text{)}$ immediately reacts with $\text{NO}\text{)}$ generating cytotoxic $\text{ONOO}^-\text{)}$ and this reaction itself has several consequences. First, $\text{ONOO}^-\text{)}$ alters function of biomolecules by protein nitration as well as causing lipid peroxidation (Turko et al., 2001), i.e., potassium channels, which regulate the vasorelaxation response, are inhibited by nitration (Liu and Guttermann, 2002). Turko et al. (2001) showed that increased levels of
nitrotyrosine are associated with apoptosis of myocytes, endothelial cells and fibroblasts in diabetes. Second, ONOO• causes single-strand DNA breakage which in turn activates nuclear enzyme poly (ADP-ribose) polymerase (PARP) (Soriano et al., 2001). Third, it decreases •NO bioavailability causing impaired relaxation and inhibition of the antiproliferative effects of •NO

- Furthermore, ONOO• oxidizes tetrahydrobiopterin (BH4), an important cofactor for NOS and causes uncoupling of NOS, which produces •O2•− instead of •NO (Martin et al., 2003)

ROS-induced peroxidation of membrane lipids alters the structure and the fluidity of biological membranes, which ultimately affects function (Griendling and Fitzgerald, 2003). All these pathological modifications contribute to the pathogenesis of vascular dysfunction.

Hyperglycemia is the primary mediator of atherosclerosis in type 1 DM and intensive insulin treatment had a significant effect on serum lipid levels. Many studies have demonstrated serum lipid abnormalities in children with T1DM as well as an association between elevated HbA1c and dyslipidemia. The importance of this association emerges from that pediatric dyslipidemia is associated with atherosclerosis and requires preventive measures. As part of these preventive measures, there is indirect evidence for the importance of glycemic control in improving lipid profile in children with T1DM. Moreover, glucose is known to increase both cholesterol synthesis and OS. A recent study on forty type 1 diabetics (El-Samahy et al., 2009) a higher MDA level as a marker of lipid peroxidation was detected in diabetics with longer disease duration compared to newly diagnosed patients. They explain these findings by the fact that as the disease progresses the free radicals production is increased and the capacity of antioxidant systems is reduced. Free radicals are produced as a result of glycosylation of several proteins including hemoglobin by non-enzymatic mechanisms. Subsequently, these free radicals change lipid/protein ratio of membranes by affecting poly-unsaturated fatty acids and lipid peroxidation causing functional irregularities of several cellular organelles. Lipid peroxides are disintegrated quickly forming reactive carbon compounds. Among these, MDA is an important reactive carbon compound used commonly as an indicator of lipid peroxidation.

Importantly, there is a tight pathogenetic link between hyperglycemia-induced oxidant stress and the two hyperglycemia-dependent mechanisms of vascular damage, namely; Protein Kinase C (PKC) activation and advanced glycosylation end products (AGEs) formation Fig. 9.

![Hyperglycemia and diabetic microangiopathy](image)

Fig. 9: Hyperglycemia and diabetic microangiopathy (Aronson, 2008)
Protein Kinase C (PKC)

PKC is a family of at least 12 isoforms of serine and threonine kinases (Fig. 10). In vascular smooth muscle cells, PKC activation has been shown to modulate growth rate, DNA synthesis and growth factor receptor turnover. Hyperglycemia-induced PKC activation also results in increased platelet derived growth factor receptor expression on smooth muscle cells and other vascular wall cells. Increased expression of TGF-β is thought to lead to thickening of capillary basement membrane, one of the early structural abnormalities observed in almost all tissues in diabetes (Gould and Newton, 2008).

Advanced Glycosylation End Products (AGEs)

The effects of hyperglycemia are often irreversible and lead to progressive cell dysfunction. That cellular perturbation may persist despite the return of normoglycemia, the so-called memory effect. Thus, persistent rather than transient, acute metabolic changes are of pivotal importance in the pathogenesis of diabetic complications (Gupta et al., 2007).

One of the important mechanisms responsible for the accelerated atherosclerosis in diabetes is the nonenzymatic reaction between glucose and proteins or lipoproteins in arterial walls, collectively known as browning reaction. Glucose forms chemically reversible early glycosylation products with reactive amino groups of circulating or vessel wall proteins (Schiff bases), which subsequently rearrange to form the more stable Amadori-type early glycosylation products. Equilibrium levels of Schiff-base and Amadori products (HbA1c) are reached in hours and weeks, respectively (Gasser and Forbes, 2008).

Some of the early glycosylation products on long-lived proteins (e.g., vessel wall collagen) continue to undergo complex series of chemical rearrangement to form AGEs, once formed, AGE-protein adducts are stable and virtually irreversible. Although, AGEs comprise a large number of chemical structures, carboxymethyl-lysine-protein adducts are the predominant AGEs present in vivo (Fig. 11). AGEs accumulate continuously on long-lived vessel wall proteins with aging and at an accelerated rate in diabetes. The degree of nonenzymatic glycation is determined mainly by the glucose concentration and time of exposure (Gupta et al., 2007).
Fig. 11: Advanced glycosylation end products and the mechanism of vascular injury (Gupta et al., 2007)

Sources of OS in Diabetes

There are multiple sources of OS in diabetes including nonenzymatic, enzymatic and mitochondrial pathways.

- Nonenzymatic sources of OS originate from the oxidative biochemistry of glucose. Hyperglycemia can directly cause increased ROS generation. Glucose can undergo autoxidation and generate $\cdot OH$ radicals (Turko et al., 2001). In addition, glucose reacts with proteins in a nonenzymatic manner leading to the development of Amadori products followed by formation of AGEs. ROS is generated at multiple steps during this process. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which results in increased intracellular osmolarity, depleted glutathione reserves and enhanced production of free radicals.

- The mitochondrial respiratory chain is another source of nonenzymatic generation of reactive species. During the oxidative phosphorylation process, electrons are transferred from electron carriers NADH and FADH$_2$, through four complexes in the inner mitochondrial membrane, to oxygen, generating ATP in the process (Green et al., 2004). Under normal conditions, $\cdot O_2^-$ is immediately eliminated by natural defense mechanisms (Fig. 12). A recent study demonstrated that hyperglycemia-induced generation of $\cdot O_2^-$ at the mitochondrial level is the initial trigger of vicious cycle of oxidative stress in diabetes (Brawlee, 2005). When endothelial cells are exposed to hyperglycemia at the levels relevant to clinical diabetes, there is increased generation of ROS especially $\cdot O_2^-$ which precedes the activation of the major pathways involved in the development of diabetic complications (Fig. 13) (Santos et al., 2003).

- Enzymatic sources of augmented generation of reactive species in diabetes include NOS, NAD (P) H oxidase and xanthine oxidase. All isoforms of NOS require five cofactors/prosthetic groups such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, BH$_4$, and Ca$^{2+}$-calmodulin. If NOS lacks its substrate L-arginine or one of its cofactors, NOS may produce $\cdot O_2^-$ instead of $\cdot NO$ and this is referred to as the uncoupled state of NOS (Aliciuzel et al., 2003). -NAD (P) H oxidase
Fig. 12: Hyperglycemia increases oxidative stress (Dave and Kalia, 2007)

Fig. 13: The mitochondrial nonenzymatic sources of oxidative stress (Caro et al., 2008)

is a membrane associated enzyme that consists of five subunits and is a major source of $\cdot$O$_2$ production (Kitada et al., 2003). Its activity is significantly higher in vascular tissue obtained from diabetic patients (Ergul et al., 2004). There is possible evidence that PKC, which is stimulated in diabetes via multiple mechanisms activates NAD (P) H oxidase (Amiri et al., 2002)
Natural Defense Against Oxidative Stress and Antioxidants in Diabetes

Reactive species can be eliminated by a number of enzymatic and nonenzymatic antioxidant mechanisms. As mentioned, SOD immediately converts \( \bullet \mathrm{O}_2^- \) to \( \mathrm{H}_2\mathrm{O}_2 \), which is then detoxified to water either by catalase in the lysosomes or by glutathione peroxidase in the mitochondria. Another enzyme that is important is glutathione reductase, which regenerates glutathione that is used as a hydrogen donor by glutathione peroxidases during the elimination of \( \mathrm{H}_2\mathrm{O}_2 \). Martin and colleagues reviewed in detail that diabetes has multiple effects on the protein levels and activity of these enzymes, which further augment OS by causing a suppressed defense response.

For example:

- In the heart, which is an important target in diabetes and prone to diabetic cardiomyopathy leading to chronic heart failure, SOD and glutathione peroxides expression as well as activity are decreased whereas catalase is increased in experimental models of diabetes (Martin et al., 2003).
- In patients with chronic heart failure, all three enzymes are decreased in the smooth muscle (Linke et al., 2005). Exercise training can up-regulate the expression and activity of antioxidant enzymes.
- Increased isoprostane levels in diabetic patients with chronic heart failure are correlated with antioxidant status and disease severity (Cristina et al., 2004). Thus, modulation of these enzymes in target organs prone to diabetic complications such as heart and kidney may prove beneficial in the prevention and management of heart failure and kidney failure (Friddlyand and Philipson, 2005).
- Nonenzymatic antioxidants include vitamins A, C and E; glutathione; \( \alpha \)-lipoic acid; carotenoids; trace elements like copper, zinc and selenium; coenzyme Q\(_{10} \) (CoQ\(_{10} \)) and cofactors like folic acid, uric acid, albumin and vitamins B\(_{12} \), B\(_{6} \) and B\(_{12} \) (Fig. 14). Alterations in the antioxidant defense system in diabetes have recently been reviewed (Vega-Lopez et al., 2004).
- Glutathione (GSH) acts as a direct scavenger as well as a co-substrate for GSH peroxidase.
- Vitamin E is a fat-soluble vitamin that prevents lipid peroxidation. It exists in 8 different forms, of which \( \alpha \)-tocopherol is the most active form in humans. Hydroxyl radical reacts with tocopherol forming a stabilized phenolic radical which is reduced back to the phenol by ascorbate and NAD(P)H dependent reductase enzymes (Hersley et al., 2004).
- CoQ\(_{10} \) is an endogenously synthesized compound that acts as an electron carrier in the Complex II of the mitochondrial electron transport chain. Brownlee et al reported that this is the site of \( \bullet \mathrm{O}_2^- \) generation under hyperglycemic conditions (Brownlee, 2005). CoQ\(_{10} \) is a lipid soluble antioxidant and in higher concentrations, it scavenges \( \bullet \mathrm{O}_2^- \) and improves endothelial dysfunction in diabetes (Watts et al., 2002).
- Vitamin C (ascorbic acid) increases NO production in endothelial cells by stabilizing NO synthase cofactor BH\(_{4} \) (Heller et al., 2001).
- \( \alpha \)-Lipoic acid is a hydrophilic antioxidant and can therefore exert beneficial effects in both aqueous and lipid environments. \( \alpha \)-lipoic acid is reduced to another active compound dihydrolipoate. Dihydrolipoate is able to regenerate other antioxidants such as vitamin C, vitamin E and reduced glutathione through redox cycling (Heller et al., 2001).
In conclusion, this review highlights the role of OS and adhesion molecules in the development of diabetic MVCs and its correlation with parameters of metabolic control especially HbA1C. It allows better understanding of the early steps in the pathophysiology of developing diabetic MVCs and so giving a future hope for novel therapeutic agents preventing the early crawling diabetic vascular disease. An important issue is that these findings appear even in patients with short non-complicated diabetes course and thus provides a direct evidence for the beneficial and early effect of tight metabolic control and antioxidant therapy in prevention of MVCs.

Recommendations for achieving the appropriate metabolic control and management of dyslipidemia are the most beneficial in preventing the OS and improving endothelial function. Diet of diabetic patients should contain the recommended daily allowance of antioxidants. Starting antioxidant therapy as one of the cornerstones of treatment early after diagnosis of type 1DM may give a future hope in preventing diabetic microvascular disease.

REFERENCES


