Chapter 12
Free Radicals and Antioxidants in Human Disease

Michael Lawson, Klaudia Jomova, Patrik Poprac, Kamil Kuča, Kamil Musílek, and Marian Valko

Abstract Free radicals are species containing one or more unpaired electrons. Unpaired or free electrons are responsible for enhanced reactivity of free radicals with various biomolecules. Most frequently occurring radicals in biological systems are reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are generated by the tightly regulated enzymes, nicotinamide adenine dinucleotide phosphate oxidase isoforms and nitric oxide synthases. Overproduction of ROS and RNS results in oxidative and nitrosative stress, a state which is responsible for the damage to cell macromolecules including lipids, proteins and DNA. Oxidative stress has been implicated in the aetiology of various disease states of an organism. In this chapter, we discuss the biochemistry of free radicals and their impact on the development of various diseases. Organs of biological systems are the principal targets of oxidant species, which are implicated in atherosclerosis, diabetes, carcinogenesis and neurodegeneration. Attention is focused on oxidative stress-induced cardiovascular disease, type 2 diabetes, cancer and Alzheimer's disease. The roles of redox active metal-catalysed formation of ROS and antioxidants in protection against oxidative damage is also discussed.

Keywords Free radicals • Oxidative stress • Antioxidants • Human disease

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12.1 Introduction

The effect of reactive oxygen species (ROS) and reactive nitrogen species (RNS) causing potential biological damage is termed oxidative stress and nitrosative stress, respectively (Kovacic and Jacintho 2001; Ridnour et al. 2005; Valko et al. 2004). This state is typical for overproduction of ROS/RNS on one hand and/or a deficiency of antioxidants on the other hand. Mild oxidative stress on cells may lead to apoptosis and cells are able to overcome it by regulating the redox state. However, enhanced and prolonged oxidative stress can cause necrosis. Long-term effects of oxidative stress cause damage to cellular lipids, proteins and DNA. DNA damage by free radicals leads to formation of a variety of oxidative products. One of the most extensively studied single-base damages by oxidation is 8-hydroxy-2′-deoxyguanosine (8-oxodG) (Dizdaroglu et al. 2002). The level of 8-oxodG in urine can be regarded as a sensitive marker of oxidative stress of an organism.

Organs of biological systems are the principal targets of oxidant species, which are implicated in atherosclerosis, diabetes, carcinogenesis and neurodegeneration. Cardiovascular disease CVDs is a problem in the Western developed world. It is generally believed to be a problem of people who have a high-fat diet and inadequate regular physical exercise. However, some individuals have a genetic preposition and the disease is more common in middle age and beyond. The latter suggests that free radical damage may play a role. ROS-induced oxidative stress is believed to play some role in the cardiovascular diseases such as atherosclerosis, ischemic heart disease, hypertension, cardiomyopathies, cardiac hypertrophy and congestive heart failure (Kukreja and Hess 1992). Type 2 diabetes is generally recognized as a polygenic disease. It is believed that it develops due to a cascade of events but that the most important of these events include oxidative stress-related defects in oxidative phosphorylation machinery and mitochondrial β-oxidation leading to accumulation of intracellular triglyceride in the muscle and liver and subsequent insulin resistance (Rosca et al. 2005). β-Oxidation of long-chain fatty acids is particularly important for provision of energy in the cardiac and skeletal muscle. Changes in the expression and function of the mitochondrial inner membrane protein—uncoupling protein-2 (UCP-2)—may play an important role in pancreatic β-cell dysfunction (Krauss et al. 2003). Disturbed redox regulation as a consequence of enhanced oxidative stress has been found in various cancer cells (Milkovic et al. 2014). DNA modifications by species produced by oxidative stress mechanisms represent the first step in carcinogenesis. Mutations of genetic material appear to be a critical point not only in carcinogenesis but also in ageing. Many tumours of various origins revealed increased DNA lesions compared to normal tissues. DNA damage induced by a variety of free radicals leads to formation of more than 150 oxidized products which can now be quantified with a high level of reproducibility (Dizdaroglu et al. 2002). The most frequent modification of genetic material involves single- to double-strand breaks and pyrimidine or purine modifications which in turn may affect signalling pathways, replication errors and overall genomic instability, all forming a common denominator of cancer (Marnett 2000). The studies of Alzheimer's

disease (AD) have been directed to amyloid beta protein (Aβ) (Ow and Dunstan 2014). Monomeric Aβ is a peptide with antioxidant activity and is the main constituent of amyloid plaques in the brains of AD patients. Increased formation of Aβ linked with enhanced oxidative stress and neurotoxicity is postulated to represent a major event in the development of AD.

In this chapter, we discuss the biochemistry of free radicals and their impact on the development of CVDs, cancer, type 2 diabetes and AD. The involvement of ROS/NOS appears to be the common denominator to all these events. The roles of redox active metal-catalysed formation of ROS and antioxidants in protection against oxidative damage are also discussed. An integrated view of the sources of ROS, ROS-induced damage to main biomolecules and disease incidence is outlined in Fig. 12.1.

12.2 Free Radicals, Antioxidants and Oxidative Stress

Free radicals are species containing one or more unpaired electrons. Unpaired or free electrons are responsible for enhanced reactivity of free radicals with various biomolecules. The superoxide anion ($^{\bullet}O_2^-$) and nitric oxide (NO) are the most frequently occurring radicals in biological systems (Valko et al. 2007). Radicals derived from oxygen can be considered as the most important class of species formed in living systems. Molecular dioxygen has a specific electronic configuration; in the ground state it contains two parallel unpaired electrons in antibonding π^* orbital (Halliwell and Gutteridge 1990). Thus molecular dioxygen is a biradical with electron spin quantum number $S = 1$. Thanks to the parallel orientation of both spins of electrons, reactivity of oxygen with biomolecules (having chemical bonds formed by two electrons with antiparallel spins) is significantly reduced (Valko et al. 2004). In addition to triplet molecular dioxygen, there also exist two singlet states. In singlet states, both unpaired electrons on antibonding π^* orbitals are antiparallel. In one of the oxygen singlet states, the two antiparallel electrons are localized on different orbitals. The lifetime of such oxygen is short and very rapidly interconverts to another form of singlet oxygen ($^1O_2$), also with two antiparallel electron spins, but located on the same orbital. $^1O_2$ is the most relevant source of singlet molecular oxygen in biological systems (Cadet et al. 2000).

Addition of an electron to molecular dioxygen leads to formation of superoxide radical ($^{\bullet}O_2^-$). The added electron fills one of the half-occupied antibonding π^* orbitals. $^{\bullet}O_2^-$ is considered as a primary radical formed in various biological processes. $^{\bullet}O_2^-$ is formed by four single-electron reduction steps from molecular dioxygen into water. In addition, the major source of $^{\bullet}O_2^-$ generated in aerobic cells is via electron leakage that occurs from electron transport chains including mitochondria and the endoplasmic reticulum. $^{\bullet}O_2^-$ is produced by activated phagocytes and neutrophils in order to kill bacteria. $^{\bullet}O_2^-$ can undergo a dismutation reaction catalysed

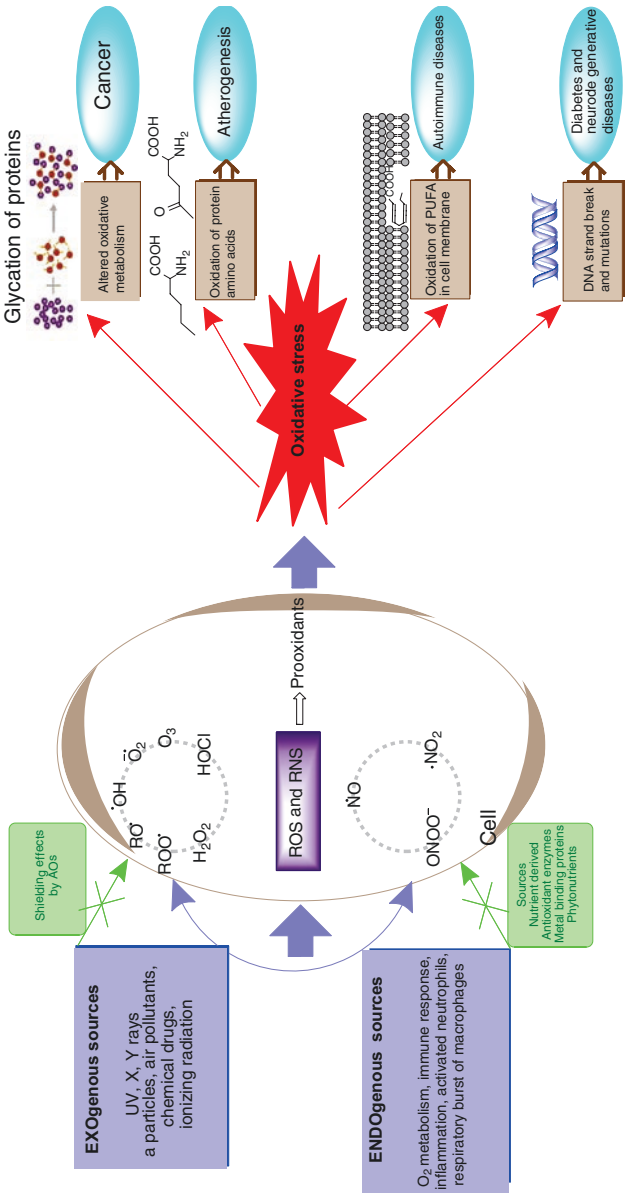

Fig. 12.1 A schematic view of the sources of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and associated oxidative damage to biomolecules and incidence of chronic diseases

by the enzyme superoxide dismutase (SOD) according to the reaction (Gutteridge et al. 1982):

$$2^{\bullet}O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

We note that the SOD enzyme works in conjunction with enzymes which remove hydrogen peroxide (H_2O_2) such as catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR).

The hydroxyl radical ($^{\bullet}OH$) is a very reactive species causing damage to all types of biomolecules. One way to generate $^{\bullet}OH$ in biological systems is via a metal-catalysed Haber-Weiss reaction (Enami et al. 2014):

$$^{\bullet}O_2^- + H_2O_2 \xrightarrow{Fe^{2+}} O_2 + {}^{\bullet}OH + OH^-$$

The Haber-Weiss reaction is an overall reaction consisting of two reactions:

$$Fe^{3+} + {}^{\bullet}O_2^- \rightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$$

The second reaction is the well-known Fenton reaction considered as a major source of $^{\bullet}OH$ in biological systems. $^{\bullet}OH$ is highly reactive with a very short half-life in aqueous solution (<1 ns). Under in vivo conditions, formation of $^{\bullet}OH$ in the proximity of important biomolecules may cause damage to them. Formation of $^{\bullet}OH$ close to DNA may cause damage to DNA bases or deoxyribosyl backbone of DNA.

The major source of $^{\bullet}O_2^-$ is from the mitochondrial electron transport chain. Ubisemiquinone is the main reductant agent of oxygen in mitochondrial membranes (Inoue et al. 2003). Mitochondria are able to produce ~2–3 nmol of $^{\bullet}O_2^-$/min per mg of protein, confirming mitochondria to be the most important physiological source of $^{\bullet}O_2^-$ in living organisms (Inoue et al. 2003). Mitochondria are highly abundant with antioxidants including the reduced glutathione (GSH), manganese SOD (Mn-SOD) and GPX, which are present on both sides of their membranes to suppress oxidative stress in the organelle (Cadenas and Davies 2000). Since $^{\bullet}O_2^-$ is negatively charged, it tends to stay in the proximity of the inner mitochondrial compartment where conversion into H_2O_2 by Mn-SOD enzyme may take place. H_2O_2 may diffuse through mitochondrial membrane (Flynn and Melov 2013) and if not eliminated (e.g. by CAT) may act as a substrate in the metal-catalysed decomposition (Fenton reaction) forming $^{\bullet}OH$. However, under physiological conditions, formation of $^{\bullet}O_2^-$ and H_2O_2 is thermodynamically rather unfavourable, and therefore we cannot expect a significant increase in $^{\bullet}O_2^-$ and H_2O_2 formation. A different situation may occur under pathological conditions, under which more profound formation of these species can be expected.

In addition to mitochondria, there exists other cellular sources of $^{\bullet}O_2^-$, including xanthine oxidase (XO) (Stein and Kirk 2015). XO is a highly versatile enzyme and

an important source of ROS. XO catalyses the reaction of hypoxanthine to xanthine and xanthine to uric acid. Additional endogenous cellular sources of ROS are neutrophils, eosinophils and macrophages. Cytochrome P450 is another source of ROS, in particular $^{\bullet}O_2^-$ and H_2O_2. Further sources of H_2O_2 include microsomes and peroxisomes.

Besides ROS, RNS play key roles in various biological processes (Gruetter et al. 1980; Bogdan 2015). Nitric oxide ($NO^{\bullet}$) contains one unpaired electron and therefore is also a radical. $NO^{\bullet}$ is an important signalling molecule in a variety of physiological processes, including regulation of blood pressure, defence mechanisms, smooth muscle relaxation and immune and neurological regulation (Ghafourifar and Cadenas 2005). $NO^{\bullet}$ is generated in biological tissues by specific nitric oxide synthases (NOSs). Overproduction of RNS is called nitrosative stress (Giuffrè et al. 2014). This may occur when the generation of RNS in a system exceeds the system's ability to neutralize and eliminate them. Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function. Cells of the immune system produce both the $^{\bullet}O_2^-$ and $NO^{\bullet}$ during the oxidative burst triggered during inflammatory processes. Under these conditions, $NO^{\bullet}$ and $^{\bullet}O_2^-$ may react together forming the very reactive peroxynitrite anion ($ONOO^-$) (Carr et al. 2000):

$$NO^{\bullet} + {}^{\bullet}O_2^- \rightarrow ONOO^-$$

This reaction proceeds very fast and the product of the reaction can cause DNA damage and peroxidation of lipids (Carr et al. 2000). The harmful effect of ROS and RNS is suppressed by the action of enzymatic antioxidants as well as by small molecular weight antioxidants. The most efficient antioxidant enzymes involve SOD, CAT and GPX. SOD exists in several isoforms, cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD and extracellular SOD (EC-SOD) (Landis and Tower 2005). As already discussed above, SOD converts $^{\bullet}O_2^-$ into H_2O_2 at the enzyme active site in a "ping-pong"-type mechanism (McCord and Fridovich 2014). A different SOD enzyme that contains nickel (Ni-SOD) containing 117 amino acids was recently discovered in *Streptomyces* and cyanobacteria (Shearer and Long 2006; Shearer 2014). CAT is an enzyme located in a cell organelle called the peroxisome (Njuma et al. 2014). The enzyme is very efficient in the decomposition of H_2O_2 into water and molecular oxygen. It has been estimated that one molecule of CAT converts approximately six million molecules of H_2O_2 into water and oxygen. In various types of tumours, suppressed capacity of CAT to detoxify H_2O_2 was observed. GSH metabolism belongs to one of the most effective antioxidative defence mechanisms. There are two forms, selenium-independent glutathione *S*-transferases (GST) and selenium-dependent GPXs. Humans have four different Se-dependent GPX (Chu et al. 2004). These enzymes act by adding two electrons to reduce peroxides by forming selenoles (Se-OH). The substrate for the catalytic reaction is either H_2O_2 or an organic peroxide ROOH. Catalytic reactions can be described according to the following reactions:

$$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O$$

$$2GSH + ROOH \xrightarrow{GPx} GSSG + ROH + H_2O$$

GPX competes with CAT for H_2O_2 as a substrate and is the major source of protection against low levels of oxidative stress. GR utilizes the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) to reduce one molar equivalent oxidized glutathione disulphide (GSSG) to two molar equivalents of sulfhydryl form of GSH:

$$GSSH + NADPH \xrightarrow{GR} 2GSH + NADP^{(+)}$$

GSH is not only the major multifunctional intracellular thiol antioxidant but also the key cellular redox buffer. GSH is highly abundant in the cytosol, nuclei and mitochondria. The oxidized form of GSH is GSSG. The antioxidant activity of all thiol compounds including GSH is their ability to accommodate a single electron originating from various types of radicals forming thiyl radicals ($GS^{\bullet}$) with much longer lifetime than $R^{\bullet}$ and reduced reactivity:

$$GSH + R^{\bullet} \rightarrow GS^{\bullet} + RH$$

GSSG is accumulated inside the cells and the ratio of GSH/GSSG is a good measure of oxidative stress of living systems. GSH reacts with $^{\bullet}OH$ and $^1O_2$ and regenerates important low-molecular-weight antioxidants. Decreased levels of GSH have been linked to a number of disease states of an organism including cancer; neurodegenerative, CVDs and pulmonary disease; HIV infection; acute pancreatitis; and others (Pastore et al. 2003).

12.3 Dietary Antioxidants

Vitamin C (ascorbic acid) is one of the most important and powerful antioxidants that acts in aqueous environments of the body. Vitamin C coacts with vitamin E to regenerate α-tocopherol from α-tocopherol radicals in membranes and lipoproteins (Chan 1993). A molecule of ascorbic acid has two ionisable hydroxyl groups. At physiological pH, the majority of vitamin C molecules are present as ascorbate anions ($AscH^-$) and only very small fraction as $AscH_2$ and Asc^{2-}. The antioxidant chemistry of vitamin C is thus the chemistry of $AscH^-$. Ascorbate anion is a donor antioxidant and reacts with radicals to form the ascorbate free radical ($Asc^{\bullet-}$) which is, due to its pK value (−0.86), not protonated and is present in the form of Asc^{2-} (Fig. 12.2). The ascorbate radical (Asc^{2-}) is considered as a poorly reactive terminal radical. The level of this radical is a good measure of the degree of oxidative stress in biological systems (Buettner 1993).

Vitamin E is a fat-soluble powerful antioxidant present in several forms. The most powerful form is α-tocopherol, which is the key membrane-bound antioxidant

(Sharma and Buettner 1993) and acts mainly against peroxidation of lipids. It has been proposed that ascorbic acid and α-tocopherol act together in a cyclic manner, in which ascorbic acid regenerates oxidized form of vitamin E (tocopherol radical, α-T-O•). The protective effect of vitamin E is substantiated by the suppressed formation of free radical formation as well as activation of endonucleases. The protective effect of the intake of vitamin E supplements (200 IU/day) against colorectal cancer has been reported by an epidemiological study. The protective effect was accounted for by the triggered apoptosis of cancer cells by inducing an efficient inhibitor of cell cycles ($p21^{wafi/cip1}$).

Flavonoids represent an important group of polyphenols with more than 4000 compounds divided into 13 classes. Their common structural element is the diphenylpropane (C6-C3-C6) moiety, which consists of two aromatic rings linked through three carbon atoms (Fig. 12.3) (Terao 2009). Recent interest in flavonoids has significantly increased mainly because of their antioxidant properties and their possible beneficial effects in human health (Terao 2009). These include the treatment and prevention of cancer, CVDs and other pathological disorders. Phenolic compounds (see Fig. 12.3) may act not only as antioxidants terminating ROS but also as effective chelators of transition metal ions that are capable of catalysing lipid peroxidation. Under unphysiological conditions, e.g. high concentrations of

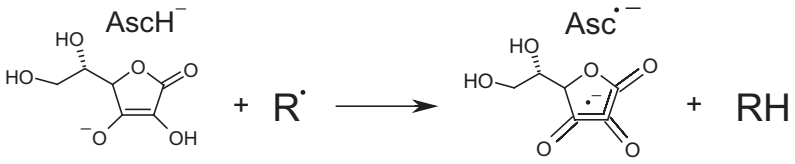

Fig. 12.2 Ascorbic acid and its reaction with radicals (R•)

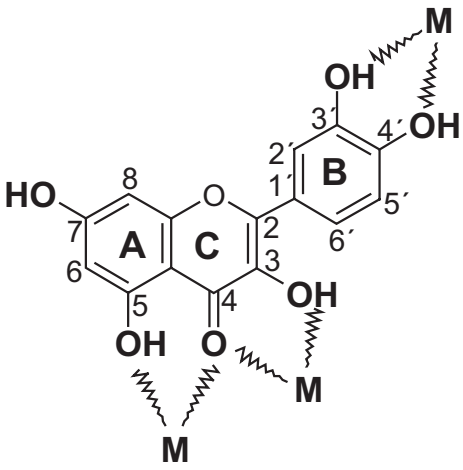

Fig. 12.3 Structure of a flavonoid quercetin. (*M* = coordinated metal ion)

flavonoids and presence of the transition metal ions, flavonoids may behave as pro-oxidants (Bast and Haenen 2013).

A regular intake of flavonoids has been associated with decreased incidence of gastrointestinal, lung and possibly breast cancers (Damianaki et al. 2000). A high intake of the flavonoid quercetin has been proposed to reduce the incidence of stroke. However, there have been studies presenting toxic effects of flavonoids as well as toxic flavonoid-drug interactions. The main symptoms were contact dermatitis, oestrogenic-related concerns and other problems. The toxic effect of flavonoids on human health has been attributed to pro-oxidant properties of flavonoids. We note that pro-oxidant effect of some antioxidants has previously been reported and was related to disturbed metabolism of redox metals as well as other unphysiological states of an organism. Thus the potential toxic effect of flavonoids under disturbed physiological conditions requires further detailed studies.

12.4 Oxidative Stress and Cardiovascular Disease

In the following, we will describe some evidence for an aetiology of CVDs due to free radical damage to cells pertinent to the healthy function of the cardiovascular system. The current most enlightening insights in the dysfunction of cardiovascular system include Ca^{2+} overload, oxidation of receptor sites, lipid peroxidation of membranes, perturbation of signalling systems and the mechanism of ischemic preconditioning. These will be described in more detail presently. Some of the substances believed to play a major role in oxidative stress in the cardiovascular system are (1) certain enzymes such as xanthine oxidoreductase (XOR), (2) NAD(P)H oxidase, (3) NOS, (4) cytochromes in mitochondria and (5) haemoglobin (Berry and Hare 2004; Hare and Stamler 2005). Biochemical pathways leading to $^{\bullet}O_2^-$ and $NO^{\bullet}$ production are given in Fig. 12.4.

ROS modify phospholipids and proteins, which lead to peroxidation and oxidation of thiol groups (Kuka et al. 2013; Molavi and Mehta 2004). Thus membrane permeability is changed with possible disruption of membrane bilayer. In addition protein function is disrupted or modified. One study (Kaneko et al. 1989) showed that oxygen free radical changes to sulfhydryl groups depressed sarcolemmal Ca^{2+} pump activities. Sarcolemma was incubated with H_2O_2 and Fe^{2+}. It was observed that ecto-ATPase activity was inhibited. The ATP-independent $Ca^{2+}$was also inhibited. After 1 min of incubation with $^{\bullet}O_2^-$, there was a 15% drop in the sarcolemmal ATP-dependent Ca^{2+} accumulation and Ca^{2+}-stimulated ATPase activities. These effects seem to correlate with an increase of malondialdehyde (MDA) in the sarcolemma. MDA is a mutagen for human cells (Niedernhofer et al. 2003) and thus will potentially further degrades cell function in this region of its occurrence. Xanthine plus xanthine oxidase or H_2O_2 caused a decrease in mitochondrial creatine kinase activity in the rat heart (Hayashi et al. 1998). They proposed that γ-glutamylcysteine ethyl ester was a suitable solution in counteracting this decrease. γ-Glutamylcysteine ethyl ester is a precursor of GSH, which is an important antioxidant but cannot be

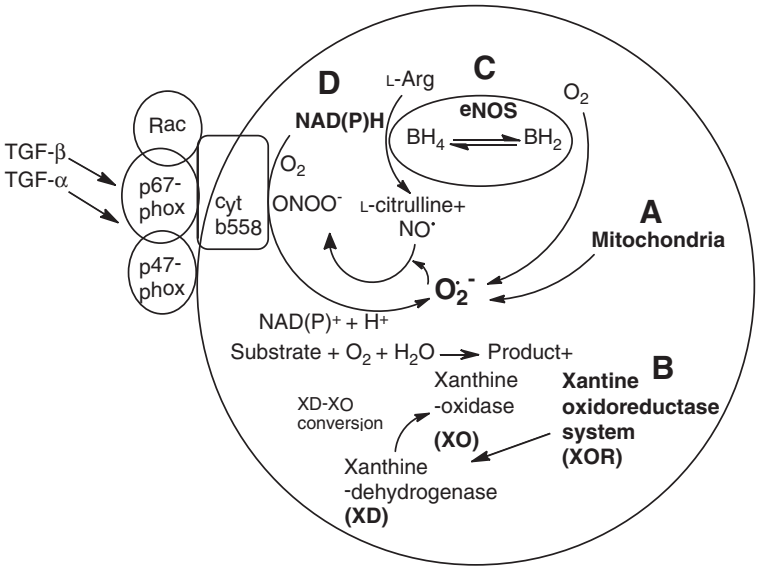

Fig. 12.4 Main pathways of ROS formation in cardiovascular system

administered directly due to degradation into constituent amino acids before entering cells (Jensen and Meister 1983).

The reduced efficiency of the Ca^{2+} pump mechanism leading to Ca^{2+} overload and resulting myocyte dysfunction has been well established in many other studies (Hua et al. 2015). ROS can have a direct effect on Ca^{2+}-handling proteins and/or membrane impairment by lipid peroxidation. This can reduce the efficiency of the pump mechanism. Stoyanovsky et al. (1997) report that $^{\bullet}O_2^-$, $^{\bullet}OH$ and $NO^{\bullet}$ promote sarcoplasmic reticular Ca^{2+} release by interaction with sulfhydryl groups of cardiac and skeletal ryanodine receptor. Other mechanisms of damage such as involving an increase in Na^+ and accumulation of long-chain fatty acids in cardiac membrane are also possible. ATP deficiency in the ischemic heart may also impair Ca^{2+} transport, and reperfusion may increase uptake of extracellular Ca^{2+} into the myocardium causing further overload. Intracellular Ca^{2+} overload appears to be associated with simulation of neointimal hyperplasia. This is believed to result in the development of atherosclerosis, vasoconstriction leading to development of hypertension, myocardial cell damage in ischemia-reperfusion and cardiac hypertrophy in heart failure.

ROS have been found to be involved in atherosclerosis. Animal models suggest large iron pools in atherosclerotic lesions. This may suggest a role of iron-catalysed formation of free radicals (Fenton reaction) in atherosclerosis (Yuan and Li 2003). Human endothelial cells show increased Ca^{2+} suggesting again Ca^{2+} overload induced oxidative stress. Uptake of oxidized low-density lipoprotein (LDLox) also seems to be important in the development of atherosclerosis (Podrez et al. 2000). It has been reported that oxidized lipoprotein and LDLox mediate enhanced $^{\bullet}O_2^-$ formation leading to apoptosis of cells in the umbilical vascular wall. LDLox-mediated

formation of ROS can cause plaque formation (Ruiz et al. 2005). Treatment with SOD and CAT can prevent these effects.

Oxidation of $NO^{\bullet}$ by $^{\bullet}O_2^-$ results in formation of $ONOO^-$, which initiates lipid peroxidation or lipoprotein oxidation. These are believed to be important events in development of atherosclerosis. Increased levels of $^{\bullet}O_2^-$ and H_2O_2 have been reported in hypertensive patients (Araujo and Wilcox 2014). $^{\bullet}O_2^-$ promotes cell proliferation while H_2O_2 induces apoptosis and activates protein kinase C. This suggests a role for protein kinase C. Hypertensive patients appear to have decreased levels of exogenous non-enzymatic antioxidants such as vitamin E and endogenous antioxidants such as GSH and SOD. Interaction of $^{\bullet}O_2^-$ and $NO^{\bullet}$ is believed to be involved in the process of hypertension (Li and Forstermann 2000). Elevated $^{\bullet}O_2^-$ levels linked with suppressed formation of $NO^{\bullet}$ from aortic rings were observed in a renal hypertension rat model. Vascular endothelial cells are known to generate $NO^{\bullet}$, so suppressed levels of this species are suggestive of endothelial dysfunction.

Angiotensin II (AngII) is a multifunctional hormone (Zucker et al. 2015). It influences cell growth, apoptosis, migration, inflammation and fibrosis (Romero and Reckelhoff 1999; Sowers 2002). It regulates blood pressure and fluid homeostasis. Evidence suggests that ROS production is tightly linked with excessive AngII-induced action. For example, AngII increases ROS by vascular smooth muscle cells. AngII-induced hypertension has also been associated with increased vascular $^{\bullet}O_2^-$ production. Angiotensin-converting enzymes (ACE) produce AngII from AngI. Since overproduction of AngII leads to induced hypertension, a possible solution is to produce inhibitors, which inhibit ACEs. AngII will appear again when we discuss $NO^{\bullet}$ signalling.

Heart tissue is rich in cardiolipin (Santucci et al. 2014). This is a phospholipid acylated in four sites, mostly with linoleic acid. It has been found that cytochrome c is normally bound to inner mitochondrial membrane (Robinson 1993). Peroxidation of cardiolipin causes dissociation of cytochrome c, which then is released through the outer mitochondrial membrane into the cytosol. The exact mechanism of cytochrome release is not clear but may involve mitochondrial permeability transition (MPT) pore with swelling of matrix and rupture of outer membrane. This is consistent with growing evidence that ROS play some role in simulating cytochrome release from mitochondria. ROS may also cause MPT by oxidation of thiol groups on adenine nucleotide translocator believed to form part of MPT pore (Petrosillo et al. 2003). Cytochrome c can also be released by mechanisms involving an oligomeric form of β-cell lymphoma-2-associated protein, an apoptosis regulator, which does not involve MPT, with no swelling and rupture of the membrane (Jürgensmeier et al. 1998).

12.5 Oxidative Stress and Type 2 Diabetes

Type 2 diabetes mellitus is an increasingly common disease in the Western world leading to increased risk to CVDs and other complications. It was formerly known as non-insulin-dependent diabetes as patients are not 100% dependent on regular

injections of insulin as are type 1 diabetes patients. It is about nine times more common than type 1 diabetes. Type 2 diabetes is believed to be due to insufficient insulin production and insulin resistance which means that certain cells, such as in the muscles, liver and fat tissue, are unable to respond appropriately, even to normal levels of insulin. This causes abnormal carbohydrate, lipid and protein metabolism. Patients may vary in the degree of inadequate insulin production, and some individuals who have insulin resistance do not develop type 2 diabetes (Gustafson et al. 2015). In insulin resistance, liver cells release too much glucose into the blood. Over prolonged periods, high glucose levels are believed to cause further deterioration in the health of the patient.

Animal models used to study diabetes usually involve rats with diabetes induced by streptozotocin, which is toxic to the insulin-producing pancreatic β-cells (Rossini et al. 1977).

It is well known that type 2 diabetes involves the dysfunction of pancreatic β-cells. Many studies have suggested that this dysfunction is a result of prolonged exposure to high glucose and elevated free fatty acid levels (Evans et al. 2003). This suggests that a high-sugar and high-fat diet is partly responsible and may perhaps be avoidable by a healthy diet.

Changes in genetic expression have also been reported to occur as a mechanism in β-cell toxicity. Robertson and colleagues suggest involvement of pancreas duodenum homobox-1 (PDX-1) and insulin gene expression (Robertson et al. 2003). Chronic exposure of HIT-T15 cells to supra-physiological concentrations of glucose over several months causes gradual loss of insulin gene expression (Robertson et al. 2003). It is thought that the mechanism involves loss of mRNA and protein levels of the PDX-1 gene. This may involve N-terminal kinase (JNK) pathway interfering with PDX-1 gene expression and is the subject of further investigation.

The role of oxidation in the formation of type 2 diabetes is particularly convincing when it was discovered that pancreatic β-cells, compared with other cell types, are low in antioxidant enzymes such as SOD, CAT and GPX (Grankvist et al. 1981). This makes them likely to be particularly sensitive to oxidative stress compared with many other types of cells. It was shown that oxidative stress by short exposure of β-cell to H_2O_2 increased production of cyclin-dependent kinase (CDK) inhibitor $p21^{WAF/CIP1/Sdi1}$ and decreased insulin mRNA, cytosolic ATP and calcium flux in cytosol and mitochondria (Kaneto et al. 1999).

The insulin receptor is composed of two extra cellular α-subunits and two transmembrane β-subunits linked by –S-S- bonds. The receptor processes an intrinsic tyrosine kinase activity. When activated by attachment of insulin to α-subunit, the receptor is phosphorylated on the tyrosine residue on the β-subunit (Lawlor and Alessi 2001). The activated receptor can then phosphorylate the insulin receptor substrate (IRS) proteins and other substrates. Phosphorylation leads to activation of different signalling pathways. The activation of phosphatidylinositol 3-kinase (PI 3-kinase) is involved in the metabolic functions of insulin. IRS1 and IRS2 are the most important substrates in insulin signalling. IRS1 can be phosphorylated on serine residues. This phosphorylation of IRS1 has a dual role either enhancing or

terminating effect of insulin. An imbalance between positive IRS1 tyrosine phosphorylation and negative IRS1 serine phosphorylation is strongly stimulated by "diabetogenetic" factors including free fatty acids, tumour necrosis factor alpha (TNFα) and oxidative stress. Insulin-activated protein kinase B (PKB) propagates insulin signalling and promotes phosphorylation of IRS1 on serine residue. This creates positive feedback for insulin action.

Insulin resistance-inducing agents such as angiotensin II, cytokines, free fatty acids, endothelin-1, cellular ROS and hyperinsulinemia can activate several serine/threonine kinases and also phosphorylate IRS1 (Vicent et al. 2003). These agents, which negatively regulate IRS1 by phosphorylation, also operate via other mechanisms such as suppression of the expression of cytokine signalling proteins (SOCS), IRS degradation and O-linked glycosylation. Clearly an understanding of the mechanisms of IRS1 inhibition and the identification of kinases involved may help in designing therapies to prevent insulin resistance.

Normally $^{\bullet}O_2^-$ is predominately produced at the sites in the mitochondrial membrane known as complex I and the ubiquinone-complex III interface. This is where long-lived intermediates allow reaction time for electrons with molecular dioxygen (Kwong and Sohal 1998). In diabetes, however, the sites are changed so that complex II becomes the main site of $^{\bullet}O_2^-$ production (Nishikawa et al. 2000). NADPH oxidase enzymes are a family of transmembrane proteins, which transport electrons across the membrane. It converts NADPH to $NADP^+$. NADPH is an electron donor and oxygen outside the cell is the electron acceptor. The result produces $^{\bullet}O_2^-$. Clearly there is potential here to be a source of ROS. Evidence supports the assertion that NADPH oxidase is a major source of glucose-induced ROS production in vascular and kidney cells suggesting NADPH as a mediator of diabetes complications (Li and Shah 2003). The cytosolic component of activated NADPH known as $p47^{phox}$ can be blocked with AngII type 1 receptor antagonists. NADPH oxidase production of ROS in diabetes can be suppressed by a variety of PKC inhibitors.

Hyperglycaemia-induced oxidative stress also occurs in nonnucleated cells (erythrocytes) lacking mitochondria and NADPH oxidase. Therefore there must be other sources of ROS production in these cases. One possible explanation is glucose autoxidation (Robertson et al. 2003). Glucose and its metabolites react with H_2O_2 in presence of iron and copper ions to form $^{\bullet}OH$. In vivo experiments have been attempted. Semchyshyn and his group (Semchyshyn et al. 2014) used intact *Saccharomyces cerevisiae* cells as in vivo model to investigate autoxidation of both glucose and fructose comparing results with in vitro experiments. They showed that in vitro fructose was more reactive than glucose and produced higher levels of autoxidation and glycation products. However, no substantive differences were observed for the effect of glucose and fructose on the intracellular level of glycoxidation products, when intact yeast cells were exposed to the high concentration of hexoses. Increases in the activities of SOD, CAT, glyoxalases and GR in both glucose- and fructose-stressed yeasts were found suggesting a reaction against the presence of oxidative/carbonyl stress. Glucose-6-phosphate dehydrogenase activity was found to decrease in yeast exposed to hexoses. Fructose was found to activate glyoxalases more than glucose.

$ONOO^-$ is highly reactive and is linked with diabetes and many other disease states (Zou et al. 2002). $ONOO^-$ reacts with the zinc cluster component of NOS so reducing the cells ability to produce $NO^\bullet$ in the places where it is desirable as a signalling molecule. Hyperglycaemia appears to be correlated with regulation of NOS expression and production of $ONOO^-$. Addition of PKC can suppress glucose-induced aortic expression of endothelial NOS (eNOS). This suggests that PKC activation is an important part of hyperglycaemia-induced NOS up-regulation, perhaps mediated by NF-κB (Hink et al. 2001).

It has been suggested that xanthine oxidase (XO) is a key source of ROS in diabetes mellitus (Butler 2000). It has been reported that the XO inhibitor allopurinol reduces oxidized lipid levels in plasma and improves blood flow in type 2 diabetes patients. Diabetes is associated with increased lipoxygenase expression, which results in eicosanoid formation. Eicosanoids are signalling molecules resulting from complex signalling pathways starting with arachidonic acid. Increased lipoxygenase expression implies these signalling pathways are overactive.

ROS and RNS deplete exogenous antioxidants as evidenced by low levels of vitamin E and vitamin C in plasma of diabetes patients. The depletion of such antioxidants leads to further oxidative damage and ROS/RNS accumulation. Hyperglycaemia and diabetes complications affect regulation of GPX expression, but the degree is somewhat variable and understanding in terms of cellular health is unclear (VanderJagt et al. 2001). The biomarkers of type 2 diabetes are MDA, GSH/GSSG ratio, S-glutathionylated proteins, F_2-isoprostanes, 3-nitrotyrosine (NO_2-Tyr) and advanced glycation end products (AGEs). The accumulation of MDA seems to play an important role in the consequences of oxidative stress in diabetes patients. One study (Wang et al. 2011) used a diabetic rat model, which showed that the administration of luteolin lowered some of the biomarkers such as MDA. The role of 4-hydroxy-nonenal (HNE) is less clear, but a few studies suggest there is accumulation of this substance in diabetes and activation of signalling pathways (Traverso et al. 2002). Isoprostanes are non-enzymatic products of arachidonic acid oxidation and are found at higher levels in plasma and urine of type 2 diabetes patients. Isoprostanes are popular as biomarkers because of specificity and sensitivity of detection. However, elevated isoprostane levels do not prove isoprostanes are a cause of the onset of diabetic complications but may be simply by-products.

Glucose is known to react directly with free amino groups in proteins and lipids to eventually form modified forms known as AGE (Ling et al. 2001). They appear to accumulate with age and also appear as biomarkers for diabetes. They can be identified in tissues by immunohistochemical techniques. They are found in many of the tissues in animal models for diabetes and type 2 diabetes patients. Certain tissues such as the liver, kidney and testis are more susceptible than others. Erythrocytes, especially the surface membranes, also contain large amounts of AGEs. AGEs can be broken down in the liver and kidneys and by macrophages. However, when there is excessive accumulation, there is tissue injury due to decreased solubility of tissue proteins. This is believed to give rise to oxidative stress in these tissues (Fu et al. 1998). It is believed that AGE formation contributes to diabetic complications and ageing in general.

The role of antioxidants in treatment of metabolic disease has been subject of various studies. In one study (Buchanan et al. 2002), treatment with troglitazone delayed or prevented onset of type 2 diabetes in high-risk Hispanic women. It is believed that this protective effect is associated with preserving pancreatic β-cell function reducing the secretory demands placed on β-cells by chronic insulin resistance. Unfortunately troglitazone has harmful effects when administered over longer periods and has been taken off the market. The low concentration of SOD, CAT and GPX in β-cells raises the evolutionary question of why this is the case. Attempts to treat diabetes with antioxidants appear to be beneficial. Antioxidant treatment appears to suppress β-cell apoptosis without causing cell proliferation. Furthermore the expression of PDX-1 was visible in nuclei of islet cells after antioxidant treatment (Kaneto et al. 1999).

12.6 Oxidative Stress and Cancer

A multistage mechanism in cancer development is characterized by multiple events occurring in a cell and can be described by three stages, initiation, promotion and progression. ROS interfere with all these three stages of carcinogenesis (Klaunig and Kamendulis 2004). Initiation stage involves DNA mutations that produce an altered cell followed by at least one round of DNA synthesis to repair the damage created during the initiation. DNA damage can be initiated by a variety of ROS, probably the most damaging being the $^{\bullet}OH$ formed via metal-catalysed Fenton reaction. An interesting direct correlation between size of tumours and the level of detected 8-oxodG adducts has been detected; thus, it may speculated that the level of oxidative adducts may be a key factor determining the transformation from benign to malignant tumours.

The typical feature of promotion stage is clonal expansion of initiated cells by the induction of cell proliferation and/or inhibition of programmed cell death (apoptosis) (Koff et al. 2015). This stage is a reversible process and requires tumour promotion stimulus. During this stage, a strong inhibition of antioxidant pool such as SOD and CAT has been observed. The ROS formation during this stage is the main line of ROS-induced promotion of tumour growth. The third and final stage of carcinogenesis is irreversible and involves molecular changes accompanying the transformation of cells from the preneoplastic to the neoplastic state. Genetic damage and breakage of chromosome integrity are typical features of this stage (see Fig. 12.5).

As already discussed above Mn-SOD catalyses the dismutation reaction. The role of Mn-SOD in cancer is far from clear (Behrend et al. 2003). It has been proposed that Mn-SOD may act as an indirect tumour suppressor protein. Overexpression of Mn-SOD has been related to increased levels of oxidative stress typical for cancer cells. Overexpressed Mn-SOD deplete $^{\bullet}O_2^-$ which in turn reduces ROS-mediated stimulation of cellular growth (Zhang et al. 2002). An association between cancer and various disorders of GSH-related enzyme functions has been reported (Pastore

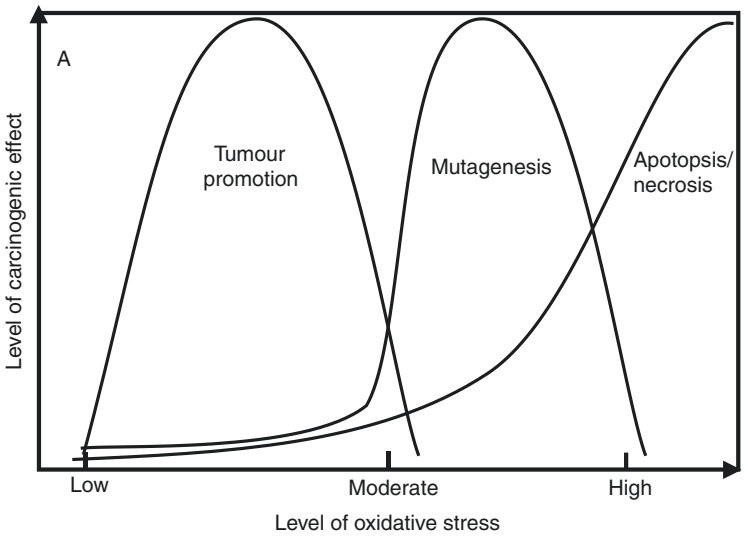

Fig. 12.5 The dependence of carcinogenic effect vs. level of oxidative stress at various stages of carcinogenic process

et al. 2003). The GSH/GSSG ratio estimated in the blood of patients with colon and breast cancers has been found to be significantly lowered compared to healthy subjects (Pastore et al. 2003). This has been clarified by an increased level of GSSG in advanced stages of cancer. This could be explained by stimulated formation of H_2O_2, which oxidizes GSH to GSSG in the red blood cells.

Low-molecular-weight antioxidants are involved in the conversion of ROS to less reactive species. However, antioxidant protection therapy in patients with advanced stages of cancer should be used very carefully, since the effects of antioxidants are strongly dependent on the stage of disease (Valko et al. 2004; Dreher and Junod 1996). Apoptosis is known to be stimulated by elevated levels of free radicals; therefore, depletion of free radicals due to the excessive administration of antioxidants might in fact stimulate survival of damaged cells and proliferation into neoplastic state and thus rather promote process of carcinogenesis than interrupt it. Antioxidant therapy during the progression stage may stimulate growth of tumours via enhanced survival of tumour cells. Pro-oxidant character of some antioxidants is also of significant importance (Mortensen et al. 2001; Valko et al. 2004).

12.7 Oxidative Stress and Alzheimer's Disease

Toxicity and oxidative stress linked with Aβ is substantiated by the disturbed metabolism of redox active metals such as iron and copper and non-redox metal zinc (Cuajungco et al. 2005). An important step in the confirmation of a significant role

of disturbed metabolism of redox active metals in Alzheimer's tissue has been made by applying three advanced physical techniques. A combination of scanning transmission ion microscopy, Rutherford backscattering spectrometry and particle-induced X-ray emission in conjunction with a high-energy (MeV) proton microprobe revealed increased concentration of metals in the amyloid plaques compared with the surrounding tissues (Rajendran et al. 2009). The level of iron in amyloid plaques was found to be doubled; copper and zinc were estimated to be nearly triple of that surrounding tissue. These data document the catalytic role of transition metal ions in the formation of oxidized species which in turn contribute to the occurrence of oxidative stress in brain tissues. Copper binds to Aβ via histidine (His13, His14, His6) and tyrosine 10 (Tyr10) amino acid residues. In addition to Cu(II), Aβ also binds Zn(II) and Fe(III). Under in vitro conditions, Zn(II) precipitates Aβ. Cu(II) interaction with Aβ promotes its neurotoxicity which has been substantiated by the reduction of Cu(II) to Cu(I) and the formation of H_2O_2 (Cuajungco et al. 2000). The copper complex with Aβ has a highly positive reduction potential, characteristic of strongly reducing cupro-proteins (Huang et al. 1999).

The role of antioxidants in AD has been subject of various studies. It has been reported that Aβ stimulates copper-mediated oxidation of ascorbate (Dikalov et al. 2004). Based on this study, it was concluded that toxic Aβ peptides stimulate copper-mediated oxidation of ascorbate ($AscH^-$) and generation of $^{\bullet}OH$ which in turn may be involved in the pathogenesis of AD. The mechanism can be described as follows:

$$A\beta\text{-}Cu(II) + AscH^- \leftrightarrow A\beta\text{-}Cu(I) + Asc^{\bullet -} + H^+$$
$$A\beta\text{-}Cu(II) + Asc^{\bullet -} \leftrightarrow A\beta\text{-}Cu(I) + Asc$$
$$A\beta\text{-}Cu(I) + H_2O_2 \rightarrow A\beta\text{-}Cu(II) + {}^{\bullet}OH + OH^- \text{ (Fenton)}$$
$$A\beta\text{-}Cu(I) + O_2 \leftrightarrow A\beta\text{-}Cu(II) + {}^{\bullet}O_2^-$$

Cupric ions in the presence of H_2O_2 may catalyse ROS oxidation of the peptide via the Fenton reaction. Using electron spin resonance spectroscopy, it has been shown that the N-terminal residues of His13, His14, His6 and Tyr10 are involved in the complexation of Cu with Aβ. It has been proposed that N-terminally complexed Cu(II) is reduced by electrons originating from the C-terminal methionine residues according to a reaction (Pogocki 2003) forming the radical of Met35 ($MetS^{\bullet +}$) and reducing cupric ions to cuprous species:

$$MetS + A\beta\text{-}Cu(II) \leftrightarrow MetS^{\bullet +} + A\beta\text{-}Cu(I)$$

While from a thermodynamic point of view reduction potentials of the Cu(II)/Cu(I) and Met/$MetS^{\bullet +}$ couples are rather unfavourable, electron transfer between MetS and Aβ-Cu(II) may be accelerated by the subsequent exergonic reaction of deprotonation of $MetS^{\bullet +}$, leaving behind the 4-methylbenzyl radical, thus making the reaction viable in vivo (Pogocki 2003). The sulphide radical $MetS^{\bullet +}$ may also undergo very fast reactions with $^{\bullet}O_2^-$. This reaction is substantiated by the formation of Met-sulphoxide (MetO) which has been isolated from AD senile plagues:

$$MetS^{\bullet +} + {}^{\bullet}O_2^- \xrightarrow{\text{Met}} 2MetO$$

The amino acid methionin-35 is strongly related to the pathogenesis of AD, since this amino acid is susceptible to oxidation under vivo conditions. It has been proposed that methionin-35 oxidation to Met-sulphoxide reduced toxic and pro-apoptotic effects of the Aβ protein fragment on isolated mitochondria (Pogocki 2003). As a consequence of broken metabolism of metals, a variety of oxidative products have been detected in AD brains. They include HO-1,8-hydroxy-guanine and oxidative modification of proteins, lipids and nucleic acids. Mainly lipid peroxidations are increased in the AD brain as compared with controls. In addition, the role of metals is linked with lipid peroxidation. Markers of lipid peroxidation detected in AD brains include HNE, 4-oxo-*trans*-2-nonenal (4-ONE), acrolein and 4-oxo-*trans*-2-hexenal, all of which are well-recognized neurotoxic agents.

Vitamin C levels of plasma in patients suffering AD have been found to be decreased as compared to control patients (Sultana et al. 2013). Levels of ascorbate in cerebrospinal fluids were also found to be decreased in AD patients compared to control subjects. This may suggest suppressed reduction of α-tocopherol radical of vitamin E back to α-tocopherol. The synergistic effect of vitamins C and E was studied in AD patients (Li et al. 2012). The combination of both vitamins E and C led to the increase of vitamins E and C in plasma and cerebrospinal fluids, making them thus less prone to in vitro oxidation. However, supplementation of vitamin E alone to AD patients did not show protection against in vitro oxidation. This study confirms the importance of synergism between vitamins E and C in patients with AD.

Flavonoids have been shown to be beneficial in patients with AD (Solanki et al. 2015). Neurotoxicity induced by Aβ, whose deposition in the brain accompanies neuronal loss in AD, was shown to be attenuated in the presence of epigallocatechin gallate. Epigallocatechin gallate is currently under investigation for its role as a chemoprotective agent. In addition, catechins are of interest due to their ability to directly scavenge ROS and RNS and exert indirect antioxidant effects via activation of transcription factors and antioxidant enzymes, modulating thus the cellular redox state.

12.8 Conclusions

Oxidative stress and nitrosative stress are mediators of damage to all cellular components which in turn may lead to the development of various diseases. ROS-induced oxidative stress in cardiac and vascular myocytes has been linked with cardiovascular tissue injury. The most profound signs of oxidative stress have been noted in ischemic heart disease, atherosclerosis, hypertension, cardiomyopathies, cardiac hypertrophy and congestive heart failure. Formation of ROS modifies phospholipids and proteins leading to peroxidation and oxidation of protein thiol groups. A critical factor in CVDs is the role of intracellular Ca^{2+} overload which can be induced by direct effect of ROS on Ca^{2+}-handling proteins or indirectly, by inducing membrane lipid peroxidation. In addition, other mechanisms involving an increase in the concentration of Na^{+} and accumulation of long-chain fatty acids in cardiac membranes should be considered.

Oxidative stress has been considered to be one of the major causes of the hyperglycaemia-induced diabetes mellitus. Hyperglycaemia stimulates generation of ROS from a variety of sources. These involve oxidative phosphorylation, glucose autoxidation, NAD(P)H oxidase, lipoxygenase, cytochrome P450 monooxygenases and NOS. The role of antioxidants in treatment of metabolic disease has been subject of various studies. The low concentration of key antioxidant enzymes in β-cells was observed. In line with this, attempts to treat diabetes with antioxidants appear to be beneficial. Antioxidant treatment appears to suppress β-cell apoptosis without causing cell proliferation.

The participation of ROS at various stages of the development of cancer is evident; many issues regarding the exact role of ROS and RNS in the aetiology of multifactorial diseases such as cancer are yet to be discovered. Of key importance is to characterize qualitatively and quantitatively which product of oxidative damage would be a suitable biomarker for cancer incidence. The main signs of metal-induced oxidative damage in AD patients involve oxidative modification of proteins, lipids and nucleic acids, mainly through lipid peroxidations. In addition, the role of metals is linked with lipid peroxidation. Markers of lipid peroxidation detected in AD brains include HNE, 4-ONE, acrolein and 4-oxo-*trans*-2-hexenal, all of which are well-recognized neurotoxic agents.

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References

Araujo M, Wilcox CS. Oxidative stress in hypertension: role of the kidney. Antioxid Redox Signal. 2014;20:74–101.

Bast A, Haenen GR. Ten misconceptions about antioxidants. Trends Pharmacol Sci. 2013;34: 430–6.

Behrend L, Henderson G, Zwacka RM. Reactive oxygen species in oncogenic transformation. Biochem Soc Trans. 2003;31:1441–4.

Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J Physiol. 2004;555:589–606.

Bogdan C. Nitric oxide synthase in innate and adaptive immunity: an update. Trends Immunol. 2015;36:161–78.

Buchanan TA, Xiang AH, Peters RK, Kjos SL, Marroquin A, Goico J, Ochoa C, et al. Preservation of pancreatic beta-cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk Hispanic women. Diabetes. 2002;51:2796–803.

Buettner GR. The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. Arch Biochem Biophys. 1993;300:535–43.

Butler J. Thermodynamic considerations of free radical reactions. In: Rhodes CJ, editor. Toxicology of the human environment. London: Taylor and Francis; 2000. p. 437–53.

Cadenas E, Davies KJA. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med. 2000;29:222–30.

Cadet J, Douki T, Pouget JP, Ravanat JL. Singlet oxygen DNA damage products: formation and measurement. Methods Enzymol. 2000;319:143–53.

Carr AC, McCall MR, Frei B. Oxidation of LDL by myeloperoxidase and reactive nitrogen species - reaction pathways and antioxidant protection. Arterioscler Thromb Vasc Biol. 2000;20:1716–23.

Chan AC. Partners in defense, vitamin E and vitamin C. Can J Physiol Pharmacol. 1993;71:725–31.

Chu FF, Esworthy RS, Doroshow JH. Role of Se-dependent glutathione peroxidases in gastrointestinal inflammation and cancer. Free Radic Biol Med. 2004;36:1481–95.

Cuajungco MP, Goldstein LE, Nunomura A, Smith MA, Lim JT, Atwood CS, Huang X, et al. Evidence that the beta-amyloid plaques of Alzheimer's disease represent the redox-silencing and entombment of abeta by zinc. J Biol Chem. 2000;275:19439–42.

Cuajungco MP, Frederickson CJ, Bush AI. Amyloid-beta metal interaction and metal chelation. Subcell Biochem. 2005;38:235–54.

Damianaki A, Bakogeorgou E, Kampa M, Notas G, Hatzoglou A, Panagiotou S, Gemetzi C, et al. Potent inhibitory action of red wine polyphenolson human breast cancer cells. J Cell Biochem. 2000;78:429–41.

Dikalov SI, Vitek MP, Mason RP. Cupric-amyloid beta peptide complex stimulates oxidation of ascorbate and generation of hydroxyl radical. Free Radic Biol Med. 2004;36:340–7.

Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H. Free radical-induced damage to DNA: mechanisms and measurement. Free Radic Biol Med. 2002;32:1102–15.

Dreher D, Junod AF. Role of oxygen free radicals in cancer development. Eur J Cancer. 1996;32A:30–8.

Enami S, Sakamoto Y, Colussi AJ. Fenton chemistry at aqueous interfaces. Proc Natl Acad Sci U S A. 2014;111:623–8.

Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? Diabetes. 2003;52:1–8.

Flynn JM, Melov S. SOD2 in mitochondrial dysfunction and neurodegeneration. Free Radic Biol Med. 2013;62:4–12.

Fu S, Fu MX, Baynes JW, Thorpe SR, Dean RT. Presence of dopa and amino acid hydroperoxides in proteins modified with advanced glycation end products (AGEs): amino acid oxidation products as a possible source of oxidative stress induced by AGE proteins. Biochem J. 1998;330:233–9.

Ghafourifar P, Cadenas E. Mitochondrial nitric oxide synthase. Trends Pharmacol Sci. 2005;26:190–5.

Giuffrè A, Borisov VB, Arese M, Sarti P, Forte E. Cytochrome bd oxidase and bacterial tolerance to oxidative and nitrosative stress. Biochim Biophys Acta. 2014;1837:1178–87.

Grankvist K, Marklund SL, Taljedal IB. CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione-peroxidase in pancreatic-islets and other tissues in the mouse. Biochem J. 1981;199:393–8.

Gruetter DY, Gruetter CA, Barry BK, Baricos WH, Hyman AL, Kadowitz PJ, Ignarro LJ. Activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside, and nitrosoguanidine—inhibition by calcium, lanthanum, and other cations, enhancement by thiols. Biochem Pharmacol. 1980;29:2943–50.

Gustafson B, Hedjazifar S, Gogg S, Hammarstedt A, Smith U. Insulin resistance and impaired adipogenesis. Trends Endocrinol Metab. 2015;26:193–200.

Gutteridge JM, Rowley DA, Halliwell B, Westermarck T. Increased non-protein-bound iron and decreased protection against superoxide-radical damage in cerebrospinal fluid from patients with neuronal ceroid lipofuscinoses. Lancet. 1982;2:459–60.

Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol. 1990;186:1–85.

Hare JM, Stamler JS. NO/redox disequilibrium in the failing heart and cardiovascular system. J Clin Invest. 2005;115:509–17.

Hayashi H, Iimuro M, Matsumoto Y, Kaneko M. Effects of gamma-glutamylcysteine ethyl ester on heart mitochondrial creatine kinase activity: involvement of sulfhydryl groups. Eur J Pharmacol. 1998;349:133–6.

Hink U, Li HG, Mollnau H, Oelze M, Matheis E, Hartmann M, Skatchkov M, et al. Mechanisms underlying endothelial dysfunction in diabetes mellitus. Circ Res. 2001;88:E14–22.

Hua Y, Robinson TJ, Cao Y, Shi GP, Ren J, Nair S. Cathepsin K knockout alleviates aging-induced cardiac dysfunction. Aging Cell. 2015;14:345–51.
Huang X, Cuajungco MP, Atwood CS, Hartshorn MA, Tyndall JD, Hanson GR, Stokes KC, et al. Cu(II) potentiation of Alzheimer abeta neurotoxicity. Correlation with cell-free hydrogen peroxide production and metal reduction. J Biol Chem. 1999;274:37111–6.
Inoue M, Sato EF, Nishikawa M, Park AM, Kira Y, Imada I, Utsumi K. Mitochondrial generation of reactive oxygen species and its role in aerobic life. Curr Med Chem. 2003;10:2495–505.
Jensen GL, Meister A. Radioprotection of human lymphoid cells by exogenously supplied glutathione is mediated by gamma-glutamyl transpeptidase. Proc Natl Acad Sci U S A. 1983;80:4714–7.
Jürgensmeier JM, Xie Z, Deveraux Q, Ellerby L, Bredesen D, Reed JC. Bax directly induces release of cytochrome c from isolated mitochondria. Proc Natl Acad Sci U S A. 1998;95:4997–5002.
Kaneko M, Elimban V, Dhalla NS. Mechanism for depression of heart sarcolemmal Ca2+ pump by oxygen free radicals. Am J Phys. 1989;257:H804–11.
Kaneto H, Kajimoto Y, Fujitani Y, Matsuoka T, Sakamoto K, Matsuhisa M, Yamasaki Y, et al. Oxidative stress induces p21 expression in pancreatic islet cells: possible implication in beta-cell dysfunction. Diabetologia. 1999;42:1093–7.
Klaunig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol. 2004;44:239–67.
Koff JL, Ramachandiran S, Bernal-Mizrachi L. A time to kill: targeting apoptosis in cancer. Int J Mol Sci. 2015;16:2942–55.
Kovacic P, Jacintho JD. Mechanisms of carcinogenesis: focus on oxidative stress and electron transfer. Curr Med Chem. 2001;8:773–96.
Krauss S, Zhang CY, Scorrano L, Dalgaard LT, St-Pierre J, Grey ST, , Lowell BB. Superoxide-mediated activation of uncoupling protein 2 causes pancreatic beta cell dysfunction. J Clin Invest 2003;112:1831–1842.
Kuka S, Tatarkova Z, Racay P, Lehotsky J, Dobrota D, Kaplan P. Effect of aging on formation of reactive oxygen species by mitochondria of rat heart. Gen Physiol Biophys. 2013;32:415–20.
Kukreja RC, Hess ML. The oxygen free radical system: from equations through membrane-protein interactions to cardiovascular injury and protection. Cardiovasc Res. 1992;26:641–55.
Kwong LK, Sohal RS. Substrate and site specificity of hydrogen peroxide generation in mouse mitochondria. Arch Biochem Biophys. 1998;350:118–26.
Landis GN, Tower J. Superoxide dismutase evolution and life span regulation. Mech Ageing Dev. 2005;126:365–79.
Lawlor MA, Alessi DR. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? J Cell Sci. 2001;114:2903–10.
Li HG, Forstermann U. Nitric oxide in the pathogenesis of vascular disease. J Pathol. 2000;190:244–54.
Li JM, Shah AM. ROS generation by nonphagocytic NADPH oxidase: potential relevance in diabetic nephropathy. J Am Soc Nephrol. 2003;14:S221–6.
Li FJ, Shen L, Ji HF. Dietary intakes of vitamin E, vitamin C, and β-carotene and risk of Alzheimer's disease: a meta-analysis. J Alzheimers Dis. 2012;31:253–8.
Ling X, Nagai R, Sakashita N, Takeya M, Horiuchi S, Takahashi K. Immunohistochemical distribution and quantitative biochemical detection of advanced glycation end products in fetal to adult rats and in rats with streptozotocin-induced diabetes. Labor Invest. 2001;81:845–61.
Marnett LJ. Oxyradicals and DNA damage. Carcinogenesis. 2000;21:361–70.
McCord JM, Fridovich I. Superoxide dismutases: you've come a long way, baby. Antioxid Redox Signal. 2014;20:1548–9.
Milkovic L, Siems W, Siems R, Zarkovic N. Oxidative stress and antioxidants in carcinogenesis and integrative therapy of cancer. Curr Pharm Des. 2014;20:6529–42.
Molavi B, Mehta JL. Oxidative stress in cardiovascular disease: molecular basis of its deleterious effects, its detection, and therapeutic considerations. Curr Opin Cardiol. 2004;19:488–93.
Mortensen A, Skibsted LH, Truscott TG. The interaction of dietary carotenoids with radical species. Arch Biochem Biophys. 2001;385:13–9.

Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE, Marnett LJ. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. J Biol Chem. 2003;278:31426–33.
Nishikawa T, Edelstei D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature. 2000;404:787–90.
Njuma OJ, Ndontsa EN, Goodwin DC. Catalase in peroxidase clothing: interdependent cooperation of two cofactors in the catalytic versatility of KatG. Arch Biochem Biophys. 2014;544:27–39.
Ow SY, Dunstan DE. A brief overview of amyloids and Alzheimer's disease. Protein Sci. 2014;23:1315–31.
Pastore A, Federici G, Bertini E, Piemonte F. Analysis of glutathione: implication in redox and detoxification. Clin Chim Acta. 2003;333:19–39.
Petrosillo G, Ruggiero FM, Paradies G. Role of reactive oxygen species and cardiolipin in the release of cytochrome c from mitochondria. FASEB J. 2003;17:2202–8.
Podrez EA, Abu-Soud HM, Hazen SL. Myeloperoxidase-generated oxidants and atherosclerosis. Free Radic Biol Med. 2000;28:1717–25.
Pogocki D. Alzheimer's beta-amyloid peptide as a source of neurotoxic free radicals: the role of structural effects. Acta Neurobiol Exp. 2003;63:131–45.
Rajendran R, Minqin R, Ynsa MD, Casadesus G, Smith MA, Perry G, Halliwell B, et al. A novel approach to the identification and quantitative elemental analysis of amyloid deposits—insights into the pathology of Alzheimer's disease. Biochem Biophys Res Commun. 2009;382:91–5.
Ridnour LA, Isenberg JS, Espey MG, Thomas DD, Roberts DD, Wink DA. Nitric oxide regulates angiogenesis through a functional switch involving thrombospondin-1. Proc Natl Acad Sci U S A. 2005;102:13147–52.
Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H. Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. Diabetes. 2003;52:581–7.
Robinson NC. Functional binding of cardiolipin to cytochrome-c-oxidase. J Bioenerg Biomembr. 1993;25:153–63.
Romero JC, Reckelhoff JF. State-of-the-art lecture. Role of angiotensin and oxidative stress in essential hypertension. Hypertension. 1999;34:943–9.
Rosca MG, Mustata TG, Kinter MT, Ozdemir AM, Kern TS, Szweda LI, Brownlee M, et al. Glycation of mitochondrial proteins from diabetic rat kidney is associated with excess superoxide formation. Am J Physiol Renal Physiol. 2005;289:F420–30.
Rossini AA, Like AA, Dulin WE, Cahill GF Jr. Pancreatic beta cell toxicity by streptozotocin anomers. Diabetes. 1977;26:1120–4.
Ruiz MC, Medina A, Moreno JM, Gómez I, Ruiz N, Bueno P, Asencio C, et al. Relationship between oxidative stress parameters and atherosclerotic signs in the carotid artery of stable renal transplant patients. Transplant Proc. 2005;37:3796–8.
Santucci R, Sinibaldi F, Polticelli F, Fiorucci L. Role of cardiolipin in mitochondrial diseases and apoptosis. Curr Med Chem. 2014;21:2702–14.
Semchyshyn HM, Miedzobrodzki J, Bayliak MM, Lozinska LM, Homza BV. Fructose compared with glucose is more a potent glycoxidation agent in vitro, but not under carbohydrate-induced stress in vivo: potential role of antioxidant and antiglycation enzymes. Carbohydr Res. 2014;384:61–9.
Sharma MK, Buettner GR. Interaction of vitamin C and vitamin E during free radical stress in plasma: an ESR study. Free Radic Biol Med. 1993;14:649–53.
Shearer J. Insight into the structure and mechanism of nickel-containing superoxide dismutase derived from peptide-based mimics. Acc Chem Res. 2014;47:2332–41.
Shearer J, Long LM. A nickel superoxide dismutase maquette that reproduces the spectroscopic and functional properties of the metalloenzyme. Inorg Chem. 2006;45:2358–60.
Solanki I, Parihar P, Mansuri ML, Parihar MS. Flavonoid-based therapies in the early management of neurodegenerative diseases. Adv Nutr. 2015;6:64–72.
Sowers JR. Hypertension, angiotensin II, and oxidative stress. N Engl J Med. 2002;346:1999–2001.
Stein BW, Kirk ML. Electronic structure contributions to reactivity in xanthine oxidase family enzymes. J Biol Inorg Chem. 2015;20:183–94.

Stoyanovsky D, Murphy T, Anno PR, Kim YM, Salama G. Nitric oxide activates skeletal and cardiac ryanodine receptors. Cell Calcium. 1997;21:19–29.
Sultana R, Perluigi M, Allan Butterfield D. Lipid peroxidation triggers neurodegeneration: a redox proteomics view into the Alzheimer disease brain. Free Radic Biol Med. 2013;62:157–69.
Terao J. Dietary flavonoids as antioxidants. Forum Nutr. 2009;61:87–94.
Traverso N, Menini S, Odetti P, Pronzato MA, Cottalasso D, Marinari UM. Diabetes impairs the enzymatic disposal of 4-hydroxynonenal in rat liver. Free Radic Biol Med. 2002;32:350–9.
Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem. 2004;266:37–56.
Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44–84.
VanderJagt DJ, Harrison JM, Ratliff DM, Hunsaker LA, Vander Jagt DL. Oxidative stress indices in IDDM subjects with and without long-term diabetic complications. Clin Biochem. 2001;34:265–70.
Vicent D, Ilany J, Kondo T, Naruse K, Fisher SJ, Kisanuki YY, Bursell S, et al. The role of endothelial insulin signaling in the regulation of vascular tone and insulin resistance. J Clin Invest. 2003;111:1373–80.
Wang GG, XH L, Li W, Zhao X, Zhang C. Protective effects of luteolin on diabetic nephropathy in STZ-induced diabetic rats. Evid Based Complement Alternat Med. 2011:Article number 323171.
Yuan XM, Li W. The iron hypothesis of atherosclerosis and its clinical impact. Ann Med. 2003;35:578–91.
Zhang Y, Zhao W, Zhang HJ, Domann FE, Oberley LW. Overexpression of copper zinc superoxide dismutase suppresses human glioma cell growth. Cancer Res. 2002;62:1205–12.
Zou MH, Shi CM, Cohen RA. Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. J Clin Investig. 2002;109:817–26.
Zucker IH, Schultz HD, Patel KP, Wang HJ. Modulation of angiotensin II signaling following exercise training in heart failure. Am J Physiol Heart Circ Physiol. 2015;308:H781–91.