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OXIDATIVE STRESS IMPLICATIONS FOR THE PATHOGENESIS OF OCULAR PATHOLOGY

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ABSTRACT

The eyes are at particular risk for oxidative damage due to their high exposure to oxygen, a large amount of fatty acids in the retina and also high light exposure, environmental pollutants and ultraviolet rays. Oxidative stress to the largely retinal pigment epithelial cell layer (RPE) over time is reported to produce tissue dysfunction that contributes to the development of the pathogenesis of many diseases of the visual apparatus. The present paper discussed the evidence found about the possible role of oxidative damage in the pathogenesis of several eye diseases (glaucoma, cataract, diabetic retinopathy, macular degeneration) and the role of diet and antioxidant supplements in the prevention and treatment of such diseases. In recent years it has been suggested that free radicals and oxidative stress are part of this process, which fact is confirmed in many instances, it has been shown that the use of exogenous antioxidants preventive or stimulation of endogenous antioxidant systems retard appearance of the main signs and symptoms of ocular pathologies.

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INTRODUCATION

Biological redox (reduction/oxidation) reactions remain poorly understood despite their importance to most normal physiological and many pathophysiological processes (Sarsour *et al.,* 2009; Valko *et al*., 2007) Intracellular redox status, which refers to the ratio of the reversible oxidized form to the reduced form of a specific redox couple, maintains cellular homeostasis through the balance of oxidants and antioxidants. Redox reactions normally regulate vascular tone (Faraci *et al*., 2006), platelet activation (Freedman *et al*., 2008), and the immune response (Forman and Torres *et al*., 2002). When redox homeostasis is compromised and chronic oxidative stress persists the pathophysiological consequences involving the cardiovascular, pulmonary, renal, gastrointestinal, hepatic, and neurologic systems, as well as metabolic and inflammatory diseases. Oxidative stress can be defined as a state of imbalance toward the factors that generate reactive oxygen and nitrogen radicals (e.g., superoxide, hydroxyl or peroxynitrite radicals) and away from factors that protect cellular macromolecules from these reactants including antioxidants enzymatic like superoxide dismutase, catalase, and glutathione peroxidases and exogenous secondary antioxidants, such as vitamins and polyphenolic compounds. The factors that generate reactive oxygen and nitrogen species (ROS and RNS) exit as products of normal cellular physiology as well as from various exogenous sources.

Mitochondria are thought to be the source of most cellular ROS, specifically superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\cdot}$) (Hartwig *et al*., 2013). The reactions that generate ATP in the mitochondria require electrons from reduced substrates to be passed along the complex of the electron transport chain. It is estimated that 0.2-2% of the O_2 consumed in the mitochondria is univalently reduced to superoxide from complexes I and III of electron transport chain by the addition of one electron to molecular oxygen. The mitochondria produce approximately 2-3 nmol of superoxide/min per milligram of protein (Treberg *et al*., 2010). This radical is the initial and principal architect of EOS signaling and damage (Chance *et al*., 1979; Cardenas and

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Davies *et al*., 2000). Upon reacting with H_2O, superoxide can generate a perhydroxyl radical (HO_2), which has been implicated in lipid damage and protein oxidation (Cohen *et al*., 1994). However, superoxide is usually converted into H_2O_2 by the manganese-superoxide dismutase or copper/zinc-superoxide dismutase (isozymes collectively referred to as SOD) found in the mitochondrial matrix and in the intermembrane space, respectively. Reactive oxygen species (ROS) is a collective term that broadly describes a variety of molecules and free radicals (chemical species with one unpaired electron) derived from molecular oxygen: singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl. ROS have distinct biological properties, which include chemical reactivity, half-life, and lipid solubility. ROS reactivity dictates toxicity, and high reactivity results in short life, limiting diffusion (superoxide anion has a half-life of 10^{-6} s and hydroxyl radical a half-life of 10^{-9} s (Mittler *et al*., 2011).

Free radicals in living organism include hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot-}$), nitric oxide ($NO^{\cdot}$) and peroxyl ($RO_2^{\cdot}$). Peroxynitrite ($ONOO^-$), hypochlorous acid (HOCL), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and ozone (O_3) are not free radicals but can easily lead to free radicals reactions in living organism. The term “reactive oxygen species“ (ROS) is often using to include both the radical and non-radical species.Cellular ROS can be distinguished by whether they are endogenously or exogenously generated. The mitochondrial respiratory chain, the cytochrome P450 metabolic pathway, and the inflammatory response are important endogenous sources (Li *et al*., 2011). In addition to mitochondrial respiration, $O_2^{\cdot-}$ is generated by NADPH oxidases (Nox´s), xanthine oxidase, uncoupled nitric oxide synthase, lipoxygenases, and myeloperoxidase. Xanthine oxidase (XO), a highly versatile enzyme, is also an important source of oxygen-free radicals. XO catalyzes the reaction of hypoxanthine to xanthine and to uric acid by forming $O_2^{\cdot-}$ in the first step and H_2O_2 in the second step (Nishino *et al*., 2008). Immune cells including macrophages and neutrophils, as well as microsomes, generate intracellular ROS (Geeringand Simon 2011). Because ROS/RNS production is inherent to normal physiology, cells have evolved both enzymatic and nonenzymatic antioxidant defense mechanisms to scavenge radicals and to maintain redox balance.

General sources of ROS production

Mitochondria are the major source of ROS in mammals under physiological conditions. The electron transport chain (ETC) is the main source of ROS production in mitochondria (Turren *et al*., 2003). Electrons from NADH and FADH2 generated in the Krebs cycle are transferred through the ETC to reduce molecular oxygen to water, a process that involves four one-electron reduction reactions. Complex IV (cytochrome c oxidase), the terminal component of the ETC, retains all the partially reduced intermediates until full reduction of oxygen is achieved. Complex I and III are the main sources of $O_2^{\cdot-}$ production in mitochondria (Balaban *et al*., 2005). The use of O_2 as the terminal electron acceptor allows for more free energy to be generated from oxidation of nutrient. In biological oxidations a large fraction of this free energy is not released as heat but is captured in high-energy bonds such as those in ATP. Consequently, the evolutionary adoption of 0_2 as the terminal electron acceptor is thought to have made possible such fundamental biological features as multicellularity, complex nervous systems, or rapid and forceful mechanical movement. However, the benefits of aerobic metabolism carry a cost, leading to the formation of reactive oxygen species or ROS (Zimniak *et al*., 2011). An increase in ROS generation occurs when mitochondrial redox potential is significantly reduced, as happens in hypoxia, or significantly oxidized. In the latter case, the increase in ROS levels results from a depletion of antioxidant capacity as a consequence of the decrease in NADPH levels (Aon *et al*., 2010). It is generally accepted that the mitochondrial respiratory chain is the major generator of ROS in most animal cells. In addition to the mitochondrial respiratory chain and to microsomal cytochromes P450, there are other sources of ROS, e.g., NADPH oxidases (which generate superoxide), xanthine oxidase (producing $O_2^{\cdot-}$ and H_2O_2) uncoupled nitric oxide synthase (nitric oxide), lipoxygenases (fatty acid hydroperoxides), monoamine oxidase and myeloperoxidase

NADPH oxidases

NADPH oxidase (Nox) enzymes are a family of heme-containing proteins with a primary function of transporting electrons from NADPH to oxygen, producing ROS. Nox family comprises seven members, each with a distinct catalytic isoform Nox´s 1-5, dual oxidase 1 (Duox1) and dual oxidase 2 (Duox2). The expression of Nox catalytic subunits varies among different cell types and tissues/organs (Lambeth *et al*., 2004; Bedard *et al*., 2007). Emerging evidence suggest that Nox/Duox family members are important ROS producers, not only for phagocytic but also for nonphagocytic cells, although the biological functions of Nox/Duox in nonphagocytes are still mostly unknown.

The phagocyte (neutrophils and macrophages) oxidase, the first characterized NADPH oxidase, is a multicomponent complex that catalyzes the formation of $O_2^{\cdot-}$ during phagocytosis (Vignais *et al*., 2002). The phagocyte NADPH oxidase has a membrane-bound catalytic core of the enzyme, flavocytochromeb$_{558}$. The flavocytochrome is a heterodimer consisting of a large glycoprotein (Nox2), and a small protein, p22phox, and the close association of these two proteins stabilizes the flavocytochrome. During activation, multiple serine residues in the C-terminus are phosphorylated, liberating the N-terminal domain for interaction with the proline-rich region and translocation to the membrane (Huang *et al*., 1999). This allows the proline-rich activation domain in p67 phox to bind with an activation sequence in the C-terminus of Nox2 to initiate electron transfer, thus activating the enzyme (Nisimoto *et al*., 1999).

Oxidase in nonphagocytic cell has been identified with the identification of Nox1. Unlike the phagocyte oxidase, the nonphagocyte oxidases are active during normal metabolism and generate low levels of ROS even in the absence of extrinsic stimulation: however, their ROS generations are increased in response to agonist stimulation.Activated NADPH oxidases (Nox1 and Nox2) generate $O_2^{\cdot-}$ by transferring two electrons from NADPH in the cytosol to FAD (Bedard *et al*., 2007). In contrast, Nox4 predominantly produces H_2O_2 and is expressed in many types of

nonphagocytic cells located in the endoplasmic reticulum, perinuclear space and nucleus (Sturrock *et al*., 2007). ROS production from NADPH oxidases could be either extracellular or intracellular depending on the biological membranes in which the enzyme is expressed, which include plasma membrane, endosome, phagosome, endoplasmic reticulum, mitochondria, and nucleus. Nox1, Nox2, Nox4, and Nox5 can be located either at the plasma membrane or within the cell and hence can generate extracellular or intracellular ROS (Lassègue *et al*., 2010). Because excessive Nox-derived ROS contribute to the progression of a wide spectrum of diseases, the Nox family of oxidases is a highly sought after therapeutic target, and the selective blockade of individual Nox isoforms is an area of intense investigation. To date, several potential inhibitors have been identified, yet most of them seem to exhibit low selectivity, potency, and bioavailability (Jaquet *et al*., 2009).

Xanthine oxidase

Xanthine oxidase (XO) and xanthine dehydrogenase (XDH) are interconvertibleisozymes of the enzyme xanthine oxidoreductase (XOR) and catalyze the final two steps of the purine (adenosine) degradation pathway, reducing hypoxanthine and xanthine to uric acid, producing $O_2^{\cdot-}$ and H_2O_2. Both forms of the enzymes act as NADH oxidases generating ROS, which may play an important role in cellular injury under conditions of increased NADH concentration (Nishino *et al*., 2008; Maia *et al*., 2005). XOR has wide tissue distribution, but its plasma levels, low in healthy mammals, increase significantly under pathophysiological conditions (Martin *et al*., 2004).

Nitric oxide synthases

The NOS family of enzymes generates $^{\cdot}NO$ from the conversion of L-arginine to L-citruline. NOSs are homodimericoxidoreductases in which the heme-containing oxygenase domain is linked to NADPH-cytochrome P450 reductase-like diflavin domain (Marletta *et al*., 1994). Upon activation, the FAD of the flavoprotein domain transfers electrons from NADPH to FMN, which reduces heme iron and results in O_2 activation followed by oxidation of guanidine N atom of L-arginine, forming NO and citruline (Channon *et al*., 2004). Three NOS isoforms are present, of which neuronal/endothelial (nNOS) and eNOS are constitutive, with activity regulated at a posttranslational level. The iNOS isoform is produced in response to proinflammatory agonists such as cytokines and is regulated mostly at the transcriptional level (Knowles and Moncada 1994; Nathan *et al*., 1995). Under normal condition eNOS exerts antiatherogenic effects in the vascular wall, leukocyte adhesion and platelet aggregation (D´Souza *et al*., 2003). NO derived from eNOS regulates muscle tone and blood pressure. However, when eNOS activity becomes "uncoupled" as happens in pathophysiological conditions (endothelial dysfunction) increased $O_2^{\cdot-}$ generation (Channon *et al*., 2004). In contract, a rapid and large increase in $^{\cdot}NO$ generation by upregulation of iNOS expression and activity was linked to cardiovascular pathology (Feng *et al*., 2001). Recent evidence indicates that nNOS has a protective function against atherosclerosis (Kuhlencordt *et al*., 2006).

Lipoxygenases

Lipoxygenases (LOXs), non-heme iron-containing dioxygenases that oxidize polyunsaturated fatty acids released from the cell membrane under inflammatory conditions to hydroperoxy fatty-acid derivatives, are another important source of ROS production. Humans have six ALOX genes (LOX genes are named "ALOX" by convention, for arachiodonic acid lipoxygenase), whereas mice have seven functional genes (Funk *et al*., 2002). LOX enzymes are named for the numbered carbon atom of the polyunsaturated fatty acid that gets oxidized (e.g., 5-LOX). 5-LOX catalyzes the transformation of free arachidonic acid to leukotriene A4, which on hydrolysis yield leukotriene B4, a potent chemoattractant and leukocyte activator (Back *et al*., 2009).

Myeloperoxidase

Myeloperoxidase (MPO) generates several oxidants that initiate lipid peroxidation and induce modification of amino acid residues in protein, including nitration, chlorination, and carbamylation(Thomas *et al*., 2008). MPO induces protein carbamylation in the presence of H_2O_2 at sites of inflammation and in atherosclerotic plaques. The proinflammatory and proatherogenic actions of MPO may include promotion of leukocyte recruitment at sites of inflammation (Klinke *et al*., 2011). Substantial evidence also suggests that MPO converts nitrite, a major end product of $^{\cdot}NO$ metabolism, into RNS, most probably nitrogen dioxide ($^{\cdot}NO_2$), in a H_2O_2 dependent reaction (Zhang *et al*., 2002). The $^{\cdot}NO_2$ generated promotes lipid peroxidation of LDL (Podrez *et al*., 1999).

Monoamine oxidase

Monoamine oxidase, existing in two isoforms (MAO A and MAO B), is a mitochondrial outer-membrane-bound flavoprotein and is another important source of mitochondrial ROS that catalyzes the deamination of neurotransmitters and biogenic amines (Edmondson *et al*., 2004).

Metal-mediated ROS generation

Metal ions produce intracellular ROS in a direct and indirect manner, where the Fenton-type reaction is one of the most well known mechanisms. During this reaction, a transition metal ion reacts with H_2O_2 to generate the highly toxic $^{\cdot}OH$ and an oxidized metal ion. Many metals, such as Fe, Cu, Cr, Co, Ni can generate free radicals via the Fenton-type reaction, although their abilities to generate free radicals differ (Desurmont *et al*., 1983). Neither the significance of the Fenton-type reaction under physiological conditions nor in vivo mechanisms by which free Fe or Cu ions mediate the generation of $^{\cdot}OH$ via the Fenton-like reaction are completely understood.

Another key mechanism in metal-induced ROS generation is the Haber-Weiss reaction. In this reaction, $O_2^{\cdot-}$ mediates $^{\cdot}OH$ generation from H_2O_2. This reaction can involve metals such as Cr and Co. The Haber-Weiss type mechanism of $^{\cdot}OH$ generation is likely to be on ROS production in the immune function of macrophages during phagocytosis (Freeman *et al*., 1982).

Role of ROS in physiological and pathological cellular functions

The formation of ROS is inevitable in aerobic organisms. By limiting the formation of ROS and bolstering anti-ROS defenses, oxidative stress can be minimized but not eliminated. A certain level of ROS is necessary because of their roles as mediators in various vital cellular processes and signaling networks (Mittler *et al*., 2011). Low level of ROS is essential for many normal cellular processes including cell signaling, cell adhesion, cellular immune response, apoptosis and cell survival (Zhu *et al*., 2012). There is a clear difference between ROS required for basic cellular mechanisms like cellular signaling and excessive ROS that contribute to oxidative stress. Free radicals and ROS have been shown to play an important part in the mammalian glucoregulatory system. For example, H_2O_2 production has been shown to regulate glucose-stimulated insulin release from β-cells and to modulate proximal and distal insulin signaling. Insulin stimulation has been shown to promote H_2O_2 production that then enhances the insulin cascade by inhibiting protein tyrosine phosphatase activity, leading to an increase in the basal phosphorylation level of insulin receptor protein (Styskal *et al*., 2012).

H_2O_2 has been linked to the redox regulation cell proliferation, apoptosis, and inflammatory processes. Some investigators suggest that H_2O_2 plays a dual role in the regulation of inflammatory processes, acting as both a proinflammatory and an anti-inflammatory agent. In addition, H_2O_2 controls opposing cellular metabolic processes such as cell proliferation, when at low concentrations, and cell death signaling at high concentration. Normal cell proliferation correlates with production of endogenous ROS through the activation of growth-related signaling pathways, including the mitogen-activated protein kinase (Andrade *et al*., 2013). Superoxide anion and hydrogen peroxide may act as signaling molecules by reaction with thiol groups of cysteine residues. The thiol/disulfide couple is ideally suited to redox modulation, serving as redox sensor and a switch to alter protein structures and/or activities. Several signaling pathways involved in cell proliferation and survival are thought to be regulated via ROS. Oxidation and reduction of thiol groups appear that on one side the redox status inside a cell is crucial for the correct functioning of many enzymes and that on the other side alterations in the redox status can serve as a signaling mechanism to activate or inactivate distinct signaling and/or DNA repair pathways (D´Autreaux and Toledano 2007).

ROS regulated cellular signaling pathways can be grouped into those affecting cell proliferation, survival, differentiation, and metabolism (e.g., thioredoxin, phosphatases); those belonging to the antioxidant and anti-inflammatory response (e. g., Nrf2/Keap 1, NF-kB); those regulating iron homeostasis (e. g., Fe-S cluster in IRP-1); and those induced in the frame of the DNA damage response (e. g., p53, ATM, PARP1, XPA) (49). For example, the apoptosis signal-regulated kinase 1 (ASK1) is an upstream mitogen activated protein kinase kinasekinase (MAPKKK) that regulates the c-Jun N-terminal kinase (JNK) and p38 MAPK pathways leading to apoptosis via phosphorylation and which is activated under conditions of oxidative stress. One mechanism of its activation consists in the oxidation of thioredoxin(Fujino *et al*., 2007). An important adaptation to oxidative stress consists in the up regulation of antioxidant detoxification genes. Two important pathways are the redox factor 1 (Ref-1)-mediated activation transcription factors and the NFE2-like 2 (Nrf2) transcription factor binding to the antioxidant-responsive element (ARE). Both pathways depend on redox regulation. In addition to the oxidative stress response, redox regulation of Ref-1/APE1 also affects basically all DNA repair systems, by activating p53, AP-1 (activator protein 1), HIF-1 α (hypoxia-inducible factor 1), and NF-kB (nuclear factor kB). Nrf2 plays a critical role in regulating expression of antioxidant and phase II drug-metabolizing enzymes, thereby contributing to detoxification and/or elimination of environmental oxidative stressors and xenobiotic and protection of cells/tissues (Zhang *et al*., 2014).

One example of a redox-regulated zinc-binding protein is the tumor suppressor p53. Tumor suppressor genes have protective functions that limit the growth of tumors and regulate many cellular activities. When tumor suppressor genes are altered, cells can grow out of control, a condition that leads to cancer. Tumor suppressor gene p53 is the most frequently mutated gene in human cancers (Vurusane *et al*., 2012) and its activation results in various cellular outcomes depending on the intensity of stress and the tissue and cellular context. P53 is weakly expressed in most cells and stabilized by escape from proteasome-mediated degradation as a response to various stress-related signals. Upon activation, it accumulates in the nucleus, binds to DNA, and regulates the transcription of many genes in addition to directly interacting with proteins involved in DNA replication, transcription, and DNA repair. Altogether, p53 provokes an antiproliferative response, including cell cycle arrest, apoptosis, DNA repair, and differentiation (Hafsi and Hainaut 2011). p53 is called the "guardian of the cell" because it trans-activates or trans-represses numerous genes to regulate cell cycle arrest, cellular senescence, and apoptosis in response to various signals. It is also activated in response to oxidative stress, in which it plays an antioxidant role (Vurusane *et al*., 2012).

There are multiple cellular processes in with both p53 and ROS are involved. Many studies have reported links between p53 and ROS. Namely, cellular ROS are increased by p53-induced transcription of pro-oxidant genes, and the pro-oxidant function of p53 has been shown to contribute to p53-induced cell death (Polyak *et al*., 1997). In addition, p53 has been implicated in the expression of some important antioxidant genes such as glutathione peroxidase and mitochondrial superoxide dismutase showing that p53 also has a protective function and participates in the antioxidant defense system. It can be suggested that different p53 target genes, including antioxidants and pro-oxidants, had opposite effects on the level of ROS. Under normal physiological conditions, low amounts of p53 suppress ROS, whereas high amounts of p53 induce ROS accumulation in response to cellular stress. Thus, these opposing responses might depend on the cellular levels of p53.While the balance between cellular antioxidant defense and ROS generation is maintained under normal metabolic conditions, excessive ROS generation can cause oxidative stress. Recent research suggests that chronic exposure to ROS causes oxidative stress by disrupting

the balance between the levels of ROS produced and the potential of cellular antioxidant systems to remove them. Prolonged and persistent oxidative stress causes activation of redox-sensitive signaling molecules. Oxidative stress also damages bio-macromolecules and eventually induces a variety of chronic and degenerative diseases (Lee *et al*., 2012).Under conditions of oxidative stress, free radicals that are not reduced or removed from the cellular environment can cause damage to all cellular macromolecules including nucleic acids, lipids, and proteins (Bokov *et al*., 2004). In general, excess amounts of ROS are detrimental and contribute to various pathologies such as atherosclerosis, heart failure, aging, diabetes, and cancer. In contrast, ROS, especially H_2O_2, at physiological levels function as signaling molecules to mediate various biological responses such as cell proliferation, migration, survival, differentiation, and gene expression (Rhee *et al*., 2000; Finkel *et al*., 2011). Cellular ROS levels are temporally and spatially regulated by the fine-tuned balance between the ROS generation system and anti-oxidant enzymes. Harmful effects of ROS on the cells include DNA damage, lipid peroxidation, protein oxidation, and inactivation of specific enzymes by oxidation of cofactors, linking to the pathological consequences. The biological effects of ROS in the cell are dependent on their amount and duration, their source and subcellular localization, and the type of species. Identifying the direct molecular target (s) of ROS in each cell type is important to understanding the cellular mechanism of redox regulation.

Lipid peroxidation-derived free aldehydes

Lipids containing polyunsaturated fatty acid (PUFAs) are susceptible to free radical-initiated oxidation and can participate in chain reactions that increase damage to biomolecules. The chain process of lipid peroxidation includes simultaneously generated lipid hydroperoxides and aldehydes of various chain lengths (Niki *et al*., 2009; Leonarduzzi *et al*., 2012). On the other hand, oxidation of lipids can cause changes in structure and fluidity of cellular and organelle membranes that are detrimental to cellular processes and functions (Esterbauer *et al*., 1993). This ultimately affects cellular functions, further increasing cellular ROS concentrations. In addition, oxidation of lipids may form lipid radical species that damage other cellular macromolecules. For example, lipid peroxides like malondialdehyde (MDA) and 4-hydroxynonenal (4HNE) can not react with both DNA and proteins (Hartley *et al*., 1997).

The enzymatic and noenzymatic peroxidation of PUFAs generates several reactive aldehyde species, which have been shown to exert regulatory roles as well as detrimental effects in various cell types and organs. These aldehydic end products of lipid peroxidation are known to be among the molecules responsible for the proatherogenic effect of oxLDL. The biological effects of oxLDLs are mediated through signaling pathways, especially involving receptors, protein kinases, and activation of transcription factors, which in turn stimulate the expression of genes involved in oxidative stress and the inflammatory response during generation of the atherosclerotic plaque (Mazièreand Mazière 2009). Indeed, oxidative stress and inflammation go hand in hand, because oxidative stress induces the production of inflammatory cytokines, and the cytokines in turn induce free radical production.The most representative unsaturated hydroxyalkenal in tissues and cells is 4HNE (Schneider *et al*., 2008). This aldehyde has been investigated in depth thanks to its contribution to the pathogenesis of major chronic human diseases, and this molecule has been reported to possess both signaling and cytotoxic effects (Poli *et al*., 2008).

Protein oxidation

Proteins in particular are susceptible to attack by numerous forms of free radicals and ROS, which can lead to many different forms of oxidative modification (Berlett and Stadtman1997). Increases in the accumulation of these forms of protein oxidative damage can lead to functional changes of protein (generally detrimental) that can alter many cellular physiological processes (Pierce *et al*., 2008). Once oxidized, proteins must be either repaired or, if repair is not possible, degraded or cleared from the cell to minimize the potential negative effects of these damaged proteins. Almost all amino acids are susceptible to oxidative modification by one or more types of ROS. The sulfur-containing amino acids (cysteine and methionine) are unique in that there are specific enzymes to repair their oxidative damage (cysteine disulfides, methionine sulfoxides). However, oxidation to other amino acids, or unresolved damage to cysteine and methionine, can result in oxidation moieties that cannot be repaired. In cases where repair is not possible, oxidized proteins are generally labeled for degradation by proteasome system or removed through autophagy processes. Despite the some system of clearance of oxidized protein, certain damaged proteins can remain and accumulate and promote cellular dysfunction (Berlett *et al*., 1997). ROS damage all the cellular macromolecules, especially proteins, because they can introduce modifications in the side chain of amino acids. These modifications can be irreversible, such as the introduction of carbonyl groups into the side chain of particular amino acids (i.e., arginine, lysine, proline and threonine). The so-called carbonylation process causes protein dysfunction and protein aggregation, leading to their accumulation during oxidative stress. Protein carboniyation has emerged as a general biomarker of protein oxidation (Nystrom *et al*., 2005; Moller *et al*., 2011, Hatem *et al*., 2014).

DNA oxidation

DNA, both nuclear and mitochondrial are susceptible to oxidation, which results in mutations and single-strand breaks along with the formation of 8-hydroxyguanosine (8-OHdG). 8-OHdG is a relatively stable oxidation product and can be measured both in tissues and in excreted urine, which accurately represent the amount of DNA oxidation/repair rate as a measure of DNA damage within the body as a whole (Wu *et al*., 2004). Oxidation of DNA has been strongly implicated in cellular senescence, apoptosis, and the development of cancerous cell phenotypes (Leonarduzzi *et al*., 2012). Especially the generation of elevated levels of DNA damage has been implicated in carcinogenicity. Oxidatively generated DNA damage includes a range of lesions such as DNA base modifications, sugar lesions, DNA single and double-strand breaks, replication errors, genomic instability, DNA-protein cross-links, and DNA-DNA cross-links. The main ROS

identified so far that lead to DNA damage are HO·, singlet oxygen ($^1O_2$), and one-electron oxidants. Among these, only HO·is able to generate DNA single-strand breaks as a consequence of initial hydrogen abstraction from the 2-deoxyribose moieties (Dedon *et al*., 2008). Concerning DNA single-base damage, $^1O_2$ reacts specifically with guanine, producing 8-0xo-7, 8-dihydroguanine (8-0xo-Gua). In addition to single-base DNA damage, HO·and one-electron oxidants have been shown to generate organic radicals which are able to react further with other DNA constituents or proteins, giving rise to more complex DNA lesions such as intra-and inter strand DNA cross-links as well as DNA-protein cross links.Transition metal ions play an important role in the induction of oxidatively damaged DNA. Whereas neither superoxide radical anion nor hydrogen peroxide is able to react with DNA directly, in the presence of transition metals such as iron, copper, cobalt or nickel H_2O_2 is converted into highly reactive HO·by Fenton-type reactions and thus induce DNA strand breaks. In metal-induced carcinogenicity, interactions with proteins involved in cell growth, apoptosis, and cellular response to DNA damage seem to be of major importance (Hartwig *et al*., 2013).

Oxidative stress and the antioxidant defense system

The aerobic organisms are protected from oxidative stress induced by reactive oxygen/nitrogen species (ROS/RNS) by an elaborate defense network in which multiple antioxidants with diverse functions play their roles. Some antioxidants are small molecules, whereas others are macromolecules such as proteins and enzymes. The physiological antioxidant systems have several lines of defense. In the first line, antioxidants prevent the production of ROS/RNS and other reactive species by, for example, sequestering active metal ions and reducing hydroperoxides and hydrogen peroxide to hydroxides and water, respectively. In the second defense line, antioxidants scavenge, quench, or remove ROS/RNS and other reactive species before they attack biological molecules. In the third defense line, antioxidant compounds and enzymes repair the damage and reconstitute membranes and tissues. Thus antioxidants act cooperatively and synergistically in the defense network to cope with oxidative stress. Furthermore, low levels of oxidative stress induce an adaptive response, which accelerates the production of antioxidant proteins and enzymes and transfers them to the right site at the right time and in the right amounts (Niki *et al*., 2014).

Biological defense against ROS comprises a complex array of endogenous antioxidant enzymes, numerous endogenous antioxidant factors including glutathione reduced and other tissue thiols, heme protein, coenzyme Q, bilirubin, urates; and a variety of nutritional factors, primarily the antioxidant vitamins. Notably, vitamin E, carotenoids and vitamin C are the essential lipophilic and hydrophilic radical-scavenging antioxidants, respectively. Because of the potential detrimental effects of oxidative stress even under normal physiological conditions, aerobic organisms have evolved a complex antioxidant system consisting of both general antioxidants (that is, those that reduce oxidative stress by removal of ROS) and specialized enzymes that can repair some forms of oxidation within cellular macromolecules. There are several enzymes involved in the antioxidant defense system.

Superoxide dismutases (Sod) reduce superoxide levels in the cell; these enzymes catalyze the conversion of the superoxide radical to molecular oxygen and hydrogen peroxide. Sod are then one of the first lines of defense against superoxide radicals produced by the mitochondria during cellular respiration and against superoxide produced by other cellular sources such as NADPH oxidases. Three main isoforms of Sod are found in mammals: CuZn superoxide dismutase CuZnSod; Sod 1), Mn superoxide dismutase (MnSod; Sod2), and extracellular superoxide dismutase (ECSod; Sod3). Each isoform is specifically localized to different cellular compartments with the primary location of Sod1 in the cytoplasm and in the mitochondrial intermembrane space, Sod2 in the mitochondrial matrix, and Sod3 in the extracellular fluids (Okado-Matsumoto and Fridovich 2001).

Peroxides, including those generated by Sod, are converted into water in the cell primarily by catalase, glutathione peroxidases, and peroxiredoxins. Catalase (Cat) is ubiquitously expressed among mammalian tissues and is primarily located in the peroxisomes. The primary catalytic function of catalase is the decomposition of hydrogen peroxide to oxygen and water (Halliwell and Gutteridge 1989). In general, glutathione peroxidases (GPx) can reduce peroxides (including hydrogen peroxide and lipid hydroperoxides) to less toxic forms including water and alcohols. There are 8 putative GPxs that differ in tissue localization and substrate specificity; however, only GPx1 and GPx4 are nearly ubiquitously expressed. Glutathione peroxidase 1 (GPx1), the most abundant isoform of the mammalian GPxs, is ubiquitously expressed, and is responsible for much of the detoxification of H_2O_2 within cytoplasm. GPx4 is ubiquitously expressed at low levels, and the specificity of GPx4 is for detoxification of lipid peroxides, including phospholipid hydroperoxides and hydroperoxides of cholesterol esters (Halliwell and Gutteridge 1989).

Peroxiredoxins (Prdx) are a relatively newly discovered class of antioxidants with peroxide activity that can reduce hydrogen peroxide, peroxynitrite, and range of different organic hydroperoxides. At least 6 different Prdx isoforms have been discovered in mammalian cells, each with specific cellular location in most cellular compartments including cytosol, nucleus, membrane, mitochondria, and Golgi (Wood *et al*., 2003). In addition, thioredoxins (Trx) catalyze reduction of disulfide bonds in multiple substrate proteins. Through this reaction, Trxs act as antioxidant by detoxifying peroxides through peroxiredoxins and by reducing protein disulfides and methionine sulfoxides (Powis and Monfort 2001). There are two forms of Trx in mammalian cells: Trx1 is located primarily in the cytosol while Trx2 is the mitochondrial form of thioredoxin. Methionine sulfoxidereductases (Msr) can also repair oxidation damage to proteins because they can catalytically reduce the oxidized form of methionine (methionine sulfoxide) back to unoxidized methionine (Stadtma *et al*., 2006). Another antioxidant defense mechanism includes nonenzymatic antioxidant such as: glutathione (GSH), uric acid, bilirubin, α- lipoic acid and nutritional factors such as: α-tocopherol (vitamin E), β-carotene, ascorbic acid (vitamin C), zinc and selenium (essential dietary component of peroxidase).

Glutathione, a tripeptide composed of cysteine, glutamate and glycine is the most abundant intracellular free thiol. GSH plays a critical role in regulating a variety of cellular functions, including detoxification of xenobiotics, synthesis of DNA and other endogenous compounds, modulation of gene expression, and regulation of the cell cycle. However, the most important and well-known function of GSH is antioxidant defense. Glutathione can reduce hydrogen peroxide and lipid peroxide through GPx-catalyzed reactions. Another important mechanism whereby GSH exerts its antioxidant function is to keep protein cysteine residues in their reduced form through reactions catalyzed by glutaredoxin and sulfiredoxin(Liu and Gaston, 2010). Cysteine residues are important for protein structure and function and they are sensitive to oxidation. Many transcription factors such as NF-kB, AP-1, and Nrf-2, as well as signaling molecules such as protein phosphatases contain redox-sensitive cysteine residues in their active sites and undergo reversible oxidative modifications upon stimulation by growth factors or oxidants. Such reversible oxidative modifications of protein cysteine residues have been increasingly recognized as an important mechanism whereby ROS/RNS regulate protein functions and cell signaling (Okamoto *et al*., 2001).

The GSH/GSSG couple is regarded as the primary arbiter of the tissue redox state because it is 2 to 4 order of magnitude higher in abundance than other redox couples and it is also metabolically linked to the less abundant redox couples via direct or indirect donations of reducing equivalents for the reduction of their oxidized forms. The key functional component of GSH is the thiol group on the cysteinyl residue, which can act both as a reductant and as a nucleophile. A unique feature of GSH oxidation/reduction reactions is that they involve two-electron transfers, whereas those of all other redox couples involve single electrons; thus, it is a highly versatile reductant, serving multiple physiological functions, including quenching of radicals by direct reactions, providing reducing equivalents for the enzyme-mediated removal of $H_2O_2$and lipid peroxides, maintenance of protein thiol groups, and conjugation and excretion of xenobiotics, among others (Lu *et al*., 2009). In various intracellular antioxidant reactions, such as removal of H_2O_2 (reaction 1), GSH is oxidized into glutathione disulfide (GSSG), which may then be excreted from the cells or reconverted to GSH by the activity of NADPH-dependent glutathione disulfide reductase (reaction 2). GSSG may also react with protein cysteinylthiolate residues to form mixed protein disulfides (reaction 3).

glutathioneperoxidase

H_2O_2. + 2GSH GSSG + 2 H_2O (1)

GSSGreductase

GSSG+ NADPH +H^+ 2GSH + $NADP^+$ (2)

Pr-S(H) + GSSG Pr-SSG + GSH (3)

As GSH is the most highly concentrated antioxidant (1 to 10 mM) in the cell and the determinant factor for cellular redox status, maintenance of intracellular GSH homeostasis is vital for normal cell functions. There are several mechanisms by which cells maintain their intracellular GSH homeostasis, including GSH redox cycling, and the novo synthesis. GSH redox cycling, catalyzed by GSSG reductase, prevents the loss of GSH in the form of GSSG that is generated during the reduction of various oxidants with GSH by reducing GSSG back to GSH (Liu and Gaston 2010). α-Lipoic acid (α-LA) is an essential cofactor of the multienzyme complexes that are associated with the mitochondrial electron transport reactions in cellular energy metabolism. α-LA and its dithiol form dihydrolipoic acid (DHLA) are also considered potent free radical scavengers and have been used to prevent or reduce reactive oxygen species-induced damage. Moreover, the α-LA-DHLA system recycles the antioxidant potency of GSH, vitamin C, vitamin E, and coenzyme Q10, thereby maintaining the cellular reduced state and countering oxidative stress. Additionally, the beneficial effects of α-LA are mediated through indirect antioxidant effects by upregulation of heme oxygenase-1 (an enzyme with antioxidant function) and may also relay on an Nrf2-dependent phase II detoxification response (Cheng *et al*., 2011).

That ascorbate (vitamin C) may serve as important antioxidant *in vivo* is widely claimed (Park and Levine 2000). The ability of ascorbic acid to show antioxidant properties is related to the fact that the dehydroascorbate radical is less reactive than are many of the radicals that can be scavenged by ascorbate(Lane *et al*., 2009). Intracellular enzymic systems exist *in vivo* to reduce this radical back to ascorbate using NADH (the NADH-semidehydroascorbatereductase enzyme) or GSH (the dehydroascorbatereductase enzyme) as sources of reducing power. Ascorbic acid is often rapidly depleted in human extracellular fluids under condition of oxidative stress (Kuiper *et al*., 2011).Vitamin E has eight isoforms, α, β, γ, and δ tocopherol and α, β, γ, and δ-tocotrienol. Tocopherols and the corresponding tocotrienols have the same scavenging capacity for free radicals. All tocopherols are antioxidants, however γ and δ are stronger antioxidants than the other because of their unmethylated carbon 5 (Traber *et al*., 2006). The relative reactivity of α, β, γ, and δ forms toward oxygen radicals decreases in order of $\alpha > \beta = \gamma > \delta$ (Yoshida *et al*., 2003; Niki *et al*., 2009; Niki *et al*., 2014). Notably, α-tocopherol has the highest bioavailability because of its highest affinity to α-tocopherol transfer protein and low rate of metabolism. α-Tocopherol, a lipid soluble antioxidant, is one of the eight vitamin E forms synthesized by plants and is the only form that meets human vitamin E requirements (Food and Nutrition Board 2000).

α-Tocopherol delays lipid peroxidation by reacting with chain propagating peroxyl radicals faster than these radicals can react with proteins of fatty acid side-chains. These antioxidants can inhibit free radical production by chelating the transition metal catysts, breaking chain reactions, reducing concentrations of ROS and by scavenging initiation radicals (Frei *et al*., 1994; Kohen *et al*., 2000; Niki and Noguchi 2004; Vlachodimitropoulou *et al*., 2010).Vitamin E scavenges active free radicals primarily by hydrogen atom transfer reaction to yield a nonradical product and vitamin E radical. Under certain conditions, vitamin E may scavenge radicals by a concerted mechanism in which an electron is transferred to yield a vitamin E cation radical, which undergoes rapid deprotonation to give a vitamin E radical. When vitamin E scavenges lipid peroxyl radical, lipid hydroperoxide and vitamin E radical are formed. The resulting vitamin E radical

may undergo several reactions: it may react with another radical to give stable products, attack lipids, or react with a reducing agent as ascorbate or ubiquinol to regenerate vitamin E. Vitamin E is not an efficient scavenger of hydroxyl radical, alkoxyl radical, nitrogen dioxide, thiyl radical, ozone, hypochlorite, and probably singlet oxygen in vivo (Niki *et al*., 2014).Vitamin E can scavenge lipid peroxyl radicals to inhibit lipid peroxidation. Vitamin E is unable to inhibit chain initiation, but it can break chain propagation. α-Tocopherol scavenges peroxil radicals during the propagation of lipid peroxidation and is termed a chain-breaking antioxidant because it prevents the chain reaction of lipid peroxidation (Niki *et al*., 2014). The effect of vitamin E as a peroxyl radical-scavenging antioxidant in vivo may be assessed from the levels of trans, trans-hydroperoxides of polyunsaturated fatty acid (PUFA), isoprostanes, neuroprostanes, 7 β-cholesterol, and 7-ketocholesterol. The inhibitory effect against lipid peroxidation depends on the concentration, distribution, and composition of lipid classes and fatty acids as well as the reactivity and concentration of antioxidant.

The reactivity of lipids toward peroxyl radical decreases in the order of PUFA >cholesterol >monounsaturated fatty acids > saturated fatty acids. The lipid classes and fatty acid composition vary markedly between tissues and possibly between individuals and they depend also on diet. Arachidonic acid and docosahexaenoic acid are the major PUFAs in the brain and retina. Vitamin E should exert beneficial effects on the inhibition of lipid peroxidation and prevention and treatment of various diseases in which free radical-mediated oxidative stress is involved, when given to the right subject at the right time and for the right duration.Dietary vitamin C, vitamin E and carotenoids provide an integrated antioxidant system with tissue GSH scavenging ROS and protecting tissues from ROS-induced oxidative damage. Once α-tocopherol reduces lipid peroxyl radicals to lipid hydroperoxides, the selenium-dependent enzyme phospholipid glutathione peroxidase (GPx4) converts the hydroperoxide to the less toxic lipid hydroxides at the expence of glutathione. Ascorbate (vitamin C) reduces the α-tocopherol radical, regenerating active α-tocopherol. Subsequently, ascorbate is regenerated at the expense of glutathione.

This ascorbate-tocopherol-GSH antioxidant system is self-regenerating at the expensive of energy (NADH, NADPH). Maintenance of this antioxidant network is crucial to protect cellular membranes against radical-mediated degradation (Lebold and Traber 2014). Cells have evolved adaptive mechanisms to endure oxidative stress. These include a battery of cytoprotective/defensive proteins that protect cells against oxidative stress and promote cell survival. Included among the cytoprotective proteins are phase II defense, such as those involved in biotransformation of xenobiotics and drugs (NAD(P)H:quinoneoxidoreductase 1 (NQO1), NRH:quinoneoxidoreductase 2 (NQO2), glutathione S-tranferase (GST), and molecules such as reduced glutathione and metallothioneins. The battery of cytoprotective proteins also includes drug transporters that play important role in drugs, intake and efflux; antiapoptotic proteins Bcl-2 and Bcl-xL, which prevent apoptotic cell death and promote cell survival; and proteasomes that remove oxidized/damage proteins (Niture *et al*., 2014).

Cytoprotective genes are ubiquitously expressed and induced in response to xenobiotics, antioxidants, oxidants, heavy metals and UV light. The induction of these genes is part of an oxidative/electrophilic stress-induced defense mechanism that includes the coordinated induction of 200+ genes. Both constitutive and inducible expression of defense genes is regulated by the antioxidant-response element (ARE) (Niture *et al*., 2014). The anatomical localization and deep yellow color of carotenoids may reduce the exposure of photoreceptor and RPE to blue light and subsequently reduce the photo-mediated production of reactive oxygen species, such as singlet oxygen. Lutein and zeaxanthin are also excellent quenchers of singlet oxygen; the capacities of lutein and zeaxanthin are superior to that of α-tocopherol. The antioxidant function of these carotenoids for protecting RPE from photo oxidation is by two different mechanisms: blocking harmful blue light and quenching reactive oxygen species. It is also known that dietary lutein and zeaxanthin play a role in modulating inflammatory response.

Supplementation of these carotenoids in RPE protected the proteasome from inactivation and attenuated the changes in expressions of these inflammation-related genes. This may be one of the mechanisms by which dietary lutein and zeaxanthin modulate ocular and systemic inflammation reduces the risk for age macular degeneration (Bian *et al*., 2012). Increasing dietary intake of grapes or lutein/zeaxanthin was sufficient to prevent RPE oxidation, cytoskeletal damage, and vision loss. This suggests that photoreceptor loss of function occurs as a consequence of oxidative damage to the RPE. The lack of benefit for normal retina function suggests that dietary antioxidants prevent the vision loss specifically caused by pathological oxidative stress rather than enhancing visual function independently. Human RPE accumulates oxidative damage with age and individuals with high oxidative burden, such as smokers, are at increased risk for macular degeneration. The long-term increase in dietary antioxidant intake probably reduces RPE oxidative damage in the human eye and may delay onset of age-related visual impairment (Yu *et al*., 2012).

Polyphenols, the most abundant antioxidants in the diet, may provide rich resources for natural medicines, possibly contributing to the prevention and/or treatment of degenerative diseases (e.g. cardiovascular disease and cancer). Polyphenols (flavonoids) may provide a means of cell or tissue protection and their antioxidant effects have been well established in vitro or in animal models. Flavonoids are polyphenolic compounds known to exert a number of biological effects, some of which may be attributed to their antioxidant properties.These compounds are a class of secondary metabolites abundantly found in plant foods (fruits, vegetables, juices and components of herbal remedies), and are the most common phenolic compounds in photosynthesizing cells. The antioxidant activity of phenolic compounds is mainly associated with three processes: 1) increases de intracellular GSH levels; 2) the blockade of Ca^{2+} influx; 3) the scavenging of free radicals and reactive species, together with inhibition of the formation and propagation of free radical reactions through the chelation of transition metal-ions. So, discovery of specific polyphenols with better

antioxidant efficacy and potency would be useful for clinical applications.

Anatomy of the eye and ocular pathology

The ocular globe is constituted by six muscles involved in the ocular movements and three concentric layers working together to provide vision, nutrition and protection to the eye. The exterior layer is constituted by the cornea and sclera. The medium or vascular layer is formed by the iris, choroid, pupil, lens and uvea. The interior layer is composed by the retina (Subczynski *et al*., 2010). The human retina is approximately 0.2 mm thick and has an area of approximately 1100mm^2. Each retina posses about 200 million neurons. The optic disc, where neuronal cells merge to form the optic nerve, is the only area of the retina that is "blind" as it lacks photoreceptors. The macula is the central posterior portion of the retina and has the highest concentration of photoreceptors, which facilitate central vision and provides highest resolution visual acuity (Bian *et al*., 2012). In the center of the macula lays the fovea, a depression with high concentration of cone cells, responsible for the central vision. An increase in dietary intake of lutein and zeaxanthin would increase the macular pigment optical density and provide better protection against photooxidation (Connolly *et al*., 2011).

Although the retina is a complex multilayered structure, it can be functionally divide in two parts: the neuronal retina, composed by photoreceptors (cones and rods) and their neuronal connections, is responsible for photo transduction process; the retinal pigment epithelium (RPE) and its basal lamina known as Bruch´s membrane maintain the integrity between retina and choroid. The RPE is composed of a polarized monolayer of pigmented hexagonal cells (melanin), and its integrity is essential for vision. Melanin in the RPE can act against ROS and protect the neural retina.The RPE is located adjacent to the outer retina, where it performs functions essential for photoreceptor survival. Its main functions include nutrient, ion, and water transport; uptake of circulating vitamin A, its storage as an ester, its conversion to retinol, and then its transfer to the photoreceptors; elimination of waste material accumulated at photoreceptors, diurnal phagocytosis and digestion of photoreceptor outer segment tips, light absorption, protection against photo-oxidation, and secretion of factors essential for maintaining the structural integrity of the retina (Bian *et al*., 2012; Yu *et al*., 2012; Burke *et al*., 2005, Strauss *et al*., 2005).

The retina is a part of the CNS, perceiving and processing visual information. But retinal photoreceptors are highly susceptible to oxidation (Tanito *et al*., 2002), because they are exposed to a range of light intensities. The RPE is at high risk for oxidative stress because it resides in a environment of high oxygen tension and is exposed to phototoxic blue light (Wu *et al*., 2006). Among the reactive oxygen species to which the cells are exposed is hydrogen peroxide. As in most cells, H_2O_2 is generated during normal oxygen metabolism in mitochondria. In the RPE, H_2O_2 is also produced during daily phagocytosis of shed photoreceptor outer segments and is generated as a consequence of light irradiation of the pigment melanin (Korytowski *et al*., 1987; Kaczara *et al*., 2010).

The eyes are at particular risk for oxidative damage due to their high exposure to oxygen, a large amount of fatty acids in the retina and also high light exposure, environmental pollutants and ultraviolet rays (Wu *et al*., 2006; Korytowski *et al*., 1987). Oxidative stress to the largely nonmitotic RPE cell layer over time is theorized to produce tissue dysfunction that contributes to the development of the pathogenesis of many diseases of the visual apparatus, such as age-related macular degeneration (AMD), diabetic retinopathy, and hereditary retinal degenerations.In mammalian tissues, the eye lens is most vulnerable to oxidative stress. The protein-rich lens depends on its ability to maintain the proteins in a reduced state by various antioxidants and oxidation defense enzymes to keep its transparency (Yu *et al*., 2013). The extensive oxidation of lens protein and lipid is associated with human cataract. A significant proportion of lenses and aqueous humor taken from cataract patients have elevated H_20_2 levels. Because H_20_2, at concentrations found in cataract, can cause lens opacification and produces a pattern of oxidation similar to that found in cataract, it is concluded that $H_20_2$is the major oxidant involved in cataract formation. In normal human eyes, it has been reported that the H_20_2 level in aqueous humor is in the range of 14-31 µM. However under pathologic conditions such as existing in cataractous eyes, the H_20_2 level in the aqueous humor differed at 33-324 µM, with a mean of 189 µM (Spector *et al*., 1998).

Oxidative stress contributes to the onset and progress of age-related macular degeneration (AMD). Due to its high metabolic rate and age-related accumulation of lipofuscin, the RPE is a primary target of photooxidative damage in the eye (Sparrow *et al*., 2005). The RPE is also a major source of cytokines that regulate inflammatory response in the retina (Bian *et al*., 2012). AMD is a multifactorial disease and leading cause of blindness in industrialized countries. Aging, genetic background, cigarette smoking, dietary factors contribute to the disease. The loss of retinal pigment epithelium with aging is related to age macular degeneration. It manifests diffuse morphologic changes at the level of the RPE, Bruch´s membrane, and photoreceptors associated with a reduction in visual acuity. These changes consist of a continuous layer of basal laminar deposit and membranous debris under the macula. AMD causes visual acuity loss by drusen, geographic atrophy, subretinal hemorrhage and serous sensory retinal detachment (Kim *et al*., 2003).

Available evidence indicates that oxidative mechanisms are involved in RPE cell death. Apoptosis is the major pathway for RPE cell death and is prevented by augmenting cellular glutathione levels through upregulation of Nrf2 activated genes. H_20_2 is released in the retina by illuminated photoreceptors and directly affected RPE cell. In the AMD eyes, the light (blue and visible) generated H_20_2 may have damage the RPE cells and then cause macular degeneration. Oxidative stress, particularly lipofuscin-mediated photooxidative damage, contributes to the progress of AMD (Bian *et al*., 2012). In the retina, docosohexanoic acid (DHA), the most abundant fatty acid in photoreceptor tips, is oxidatively modified to carboxyethylpyrrole.In the RPE, multiple proteins isolated from lipofuscin are oxidatively damaged including malondialdehyde, 4- hydroxynonenal, and AGE modifications (Sparrow *et al*., 2005).

Lipofuscin, also referred to as “aging pigment” is an aggregate containing highly oxidized and covalently cross-linked proteins (30-58%), lipids (19-51%), and even low amounts of saccharides (Jung *et al*., 2007). This material is not degradable by the proteolytic machinery of the cell nor can it be removed by exocytosis. Lipofuscin is able to catalyze and promote its own formation, both by elevating amounts of free radicals and by reducing degradation of the resulting oxidized protein that serve as precursors for lipofuscin. Another major characteristic of lipofuscin is its ability to inhibit degradation of oxidized protein by competitively binding to proteolytic enzymes including the proteasome and lysosomal proteases. Up 90% of all oxidatively damaged/modified proteins are usually degraded by the proteasomal system, a process that is essential for preservation of cellular functionality. The proteasome is involved in many aspects of cellular functions; it is involved in regulation of signal transduction and expression via controlling the levels of regulatory proteins and transcription factors. Proteosomal impairment plays a decisive role in many degenerative diseases, because of accumulation of oxidized/modified proteins that aggregate over time (Höhn *et al*., 2011).

In addition to direct oxidative damage to retina, oxidative free radicals modulate the immune-inflammatory system in part, through enhanced expression of pro-inflammatory genes. Recent studies indicate that innate immunity and inflammation are related to AMD pathogenesis. Oxidative stress and inflammation are interrelated. Whereas oxidative stress triggers inflammatory responses, inflammation also enhances the production of reactive oxygen species. The oxidative inactivation of the proteasome is a mechanistic link between oxidative stress and increased production of IL-8 by activation of the p38 MAPK signaling pathway in cultured RPE (Fernandes *et al*., 2008; Bian *et al*., 2012). The RPE has a formidable anti-oxidant defense system that must respond to its high oxidative stress environment.The ability to defend against oxidative stress by upregulating the anti-oxidant defense response is likely to be a pivotal event that mediates the initiation and progression of AMD. Due to high metabolic rate and phototoxic blue light, the RPE is a primary target of oxidative stress in the eye and subsequently may contain cellular defense mechanisms against ROS elevation. Glutathione and its related enzymes are part of this antioxidant defense (Miranda *et al*., 2010). The Age-related Eye Disease Study (AREDS) showed that anti-oxidant micronutrients reduced the progression of intermediate AMD.

Dietary lutein and zeaxanthin play significant protective roles against visual loss from AMD. Lutein and zeaxanthin in the retina may protect against AMD by two different mechanisms: blocking harmful blue light and quenching reactive oxygen (Li *et al*., 2010). Emerging evidence indicates dietary lutein and zeaxanthin have anti-inflammatory functions and reduce the risk for AMD. Supplementation with these antioxidants can partially break the vicious cycle between oxidative stress and inflammatory response in RPE cells via protecting the proteasome from inactivation and attenuate the changes in expressions of these inflammation-related genes. This may be one of the mechanisms by dietary lutein and zeaxanthin modulate ocular and systemic inflammation (Bian *et al*., 2012). Retinitis pigmentosa is a group of inherited disorders characterized by progressive photoreceptor degeneration leading to night blindness, peripheral vision loss, and subsequently central vision loss. Recently, oxidative stress has been implicated in the pathogenesis of retinitis pigmentosa. ROS are involved in numerous cellular events in the nervous system, including the retina (Halliwell *et al*., 1992). Under unfavorable circumstances, ROS may cause tremendous oxidative stress upon neurons, affect intracellular macromolecules, and lead to neuronal death in the central nervous system (CNS). The retina is a part of the CNS, perceiving and processing visual information. But retinal photoreceptors are highly susceptible to oxidation because they are exposed to a range of light intensities. Previous studies have shown that the use of combination of antioxidants (zeaxanthin, lutein, α-lipoic, acid and glutathione) drastically reduced the number of rod photoreceptors displaying oxidatively damaged DNA and delayed the degeneration processes significantly (Miranda *et al*., 2010). Komeima et al. 2006 showed that injecting another combination of antioxidants (α-tocopherol, ascorbic acid, Mn(III) tetrakisporphyrin, and α-lipoic, acid) decreased cone photoreceptor cell death in different mouse models of retinitis pigmentosa.

Diabetic retinopathy (DR) is a common complication of diabetes and a leading cause of blindness in working-age adults. Although diabetic retinopathy is considered a vascular disease, several reports demonstrate that retinal neurons are also affected, leading to vision loss (Gaspar *et al*., 2013). The degenerative changes in the retina include increases vascular permeability, leading to macular edema and endothelial cell proliferation. Diabetic retinopathy is the most frequent cause of new cases of blindness among adults aged 20 –74 years. During the first two decades of disease, nearly all patients with type 1diabetes and 60% of patients with type 2 diabetes have retinopathy.Extensive studies have shown that people with diabetic retinopathy have excess risks of systemic vascular complications, including subclinical and clinical stroke, coronary heart disease, heart failure, and nephropathy (Alghadyan *et al*., 2011).

The exact mechanism by hyperglycemia causes vascular disruption seen in retinopathy is not clear. Probably the intraocular formation of reactive oxygen species fuels the subsequent pathological, biochemical changes seen in diabetic retinopathy. These biochemical changes include: protein kinase C, glycation of proteins and polyol pathway. Oxidative stress caused by formation of free radicals as a result of hyperglycemia and the above mentioned biochemical pathways lead to damage to retinal vasculature (Fong *et al*., 2004). It was found that antioxidants such as vitamin E might prevent some of the vascular dysfunction associated with diabetes. Diabetic retinopathy progresses from mild nonproliferative abnormalities, characterized by increased vascular permeability, to moderate and severe non-proliferative diabetic retinopathy (NPDR), characterized by vascular closure, to proliferative diabetic retinopathy (PDR), characterized by the growth of new blood vessels on the retina and posterior surface of the vitreous. Macular edema, characterized by retinal thickening from leaky blood vessels, can develop at all stages of retinopathy. Glaucoma, a leading cause of irreversible blindness is the second cause of global

blindness and projections estimate that by 2020, 11 million people worldwide will be blinded by glaucoma. Progressive loss of optic nerve axons and retinal ganglion cells results in characteristic optic nerve atrophy and visual field defects in glaucoma patients (Liu *et al*., 2014). Elevated intraocular pressure triggers the initiation and progression of oxidative stress-induced toxicity resulting in programmed retinal ganglion cell death and optic nerve degeneration. Primary open-angle glaucoma (OAG) is a progressive optic neuropathy and, perhaps, the most common form of glaucoma. It is well known that OAG is a major reason for blindness, and that glaucoma is the second most important reason for blindness worldwide (Peters *et al*., 2013). Open-angle glaucoma is an asymptomatic, progressive optic neuropathy characterized by enlarging optic disc cupping and visual field loss. Elevated intraocular pressure (IOP) is a strong, modifiable risk factor for open-angle glaucoma, but it is not diagnostic. Recent evidence points to a link between increased stress and strain at the level of the optic nerve head, which somehow affects the normal function and survival of ganglion cell axons in this region.Early diagnosis depends on examination of the optic disc, retinal nerve fiber layer, and visual field.

Treatment for glaucoma consists of reducing IOP to an acceptable target range to prevent further optic nerve damage.New treatments to directly treat and protect the retinal ganglion cells that are damaged in glaucoma are also in development (Quigley *et al*., 2011). In human glaucoma patients, oxidative DNA, protein damage and lipid peroxidation has been detected in both aqueous humor and serum. Lipid aldehyde disruption of plasma membranes allows abnormal influx that can result in neuroinflammation. Additionally, glial cells exposed to ROS are potent inducers of T-cell activation, leadingto amplification of inflammatory cascades. Metal chelator co-applied therapeutic with the permeability enhancer methylsulfonylmethane (MSM) may be an effective therapeutic strategy for protecting the retina and optic nerve against oxidative damage in glaucoma. It may break the vicious cycle of oxidative stressinflammation neurodegeneration and allow endogenous antioxidant systems to eliminate accumulated ROS, repair tissue damage, and prevent neuronal death and blindness (Liu *et al*., 2014).

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