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Review

Free radicals and antioxidants: Myths, facts and mysteries

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Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are well recognized for playing a dual role as both deleterious and beneficial species. Overproduction of ROS arising either from mitochondrial electron transport chain or excessive stimulation of NAD(P)H oxidase results in oxidative stress, a deleterious process that can be an important mediator of damage to cell structures, including lipids and membranes, proteins and DNA. The powerful action of antioxidants in preventing premature lipid oxidation in food suggests that the same compounds, when consumed in the daily diet, could unfold anti-oxidative/anti-ageing effects in the human body. Failure of supplemental beta carotene to prevent cancer and cardiovascular diseases (CHD) in intervention trials, suggest that the associations of that nutrient, reflect confounding, rather than cause and effect results. The role of oxidative stress in diseases, especially cancer and CHD has been overstated. In the light of recent physiological studies, it appears more advisable to maintain the delicate internal redox balance of the cell than to interfere with the antioxidant homeostasis by a non-physiological, excessive exogenous supply of antioxidants in healthy humans.

Key words: Antioxidants, free radicals, Redox reactions, reactive oxygen species (ROS), reactive nitrogen species (RNS) and phytochemicals.

INTRODUCTION

The field of antioxidants and free radicals is often perceived as focused around the use of antioxidant supplements to prevent human diseases. In fact, antioxidants/free radicals permeate the whole of life, creating the field of redox biology. Free radicals are not all bad, neither are antioxidants all good. Life is a balance between the two: Antioxidants serve to keep down the levels of free radicals, permitting them to perform useful biological functions without too much damage (Halliwell and Gutteridge, 2006). Yet some damage is inevitable, requiring repair systems to maintain cell viability and integrity. The causes of the poisonous properties of oxygen were obscure prior to the publication of Gerschman's free radical theory of oxygen toxicity in 1954, which states that the toxicity of oxygen is due to partially reduced forms of oxygen (Gerschman et al., 1954).

In the same year, observations of a weak electron paramagnetic resonance (EPR) signal attributable to the presence of free radicals in a variety of lyophilised biological materials were reported by Commoner et al. (1954). The world of free radicals in biological systems was soon thereafter in 1956, explored by Denham Harman who proposed the concept of free radicals playing a role in the ageing process (Harman, 1956). A second era of the research on free radicals in biological systems began in 1969, when McCord and Fridovich discovered the enzyme superoxide dismutase (SOD) and

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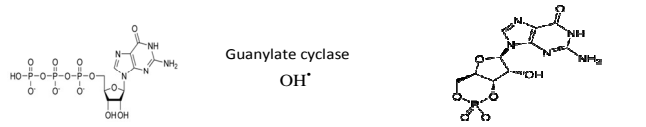
thus provided convincing evidence about the importance of free radicals in living systems (McCord and Fridovich, 1969).

A third era of free radicals in biological systems dates from 1977, when Mittal and Murad provided evidence that the hydroxyl radical, $OH^{\bullet}$, stimulates activation of guanylate cyclase and formation of the "second messenger" cyclic guanosine monophosphate (cGMP) (Mittal and Murad, 1977).



guanosine triphosphate (GTP) 3',5'-cyclic guanosine monophosphate (cGMP)

Since then, a large body of evidence has been accumulated that living systems have not only adapted to a coexistence with free radicals but have developed various mechanisms for the advantageous use of free radicals in various physiological functions. Oxygen free radicals or, more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products of normal cellular metabolism. ROS and RNS are well recognised for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems (Valko et al., 2006, 2007). Beneficial effects of ROS occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia, for example, in defense against infectious agents and in the function of a number of cellular signaling systems. One further beneficial example of ROS at low/moderate concentrations is the induction of a mitogenic response. The harmful effect of free radicals causing potential biological damage is termed oxidative stress and nitrosative stress (Kovacic and Jacintho, 2001; Ridnour et al., 2005; Valko et al., 2001). This occurs in biological systems when there is an overproduction of ROS/RNS on one side and a deficiency of enzymatic and non-enzymatic antioxidants on the other. In other words, oxidative stress results from the metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of prooxidant/antioxidant reactions in living organisms. The excess ROS can damage cellular lipids, proteins, or DNA inhibiting their normal function. Because of this, oxidative stress has been implicated in a number of human diseases as well as in the ageing process. The delicate balance between beneficial and harmful effects of free radicals is a very important aspect of living organisms and is achieved by mechanisms called "redox regulation". The process of "redox regulation" protects living organisms from various oxidative stresses and maintains "redox homeostasis" by controlling the redox status *in vivo* (Droge, 2002).

The objective of this review is to provide an insight into the production and function of ROS and to examine the effectiveness of antioxidant supplementation in human subjects.

Reactive oxygen species (ROS)

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbital (Halliwell and Gutteridge, 2006; Gutteridge and Halliwell, 2010). These unpaired electron(s) usually gives a considerable degree of reactivity to free radicals. Radicals derived from oxygen represent the most important class of radical species generated in living systems (Miller et al., 1990).

Molecular oxygen (dioxygen) has a unique electronic configuration and is itself a radical. The addition of one electron to dioxygen, forms the superoxide anion radical ($O_2^{\bullet -}$) (Miller et al., 2005) as seen in Figure 2. Superoxide anion, arising either through metabolic processes or following oxygen "activation" by physical irradiation, is considered the "primary" ROS, and can further interact with other molecules to generate "secondary" ROS, either directly or prevalently through enzyme or metal-catalysed processes (Valko et al., 2005). Various pathways of ROS formation are outlined in Figure 2. The production of superoxide occurs mostly within the mitochondria of a cell (Cadenas and Sies, 1998). The mitochondrial electron transport chain, is the main source of ATP in the mammalian cell and thus essential for life. During energy transduction, a small number of electrons leak to oxygen prematurely, forming the oxygen free radical, superoxide, which has been implicated in the pathophysiology of a variety of diseases (Kovacic et al., 2005; Valko et al., 2004). Measurements on sub-mitochondrial particles suggest an upper limit of 1 to 3% of all electrons in the transport chain leaking to generate superoxide instead of contributing to the reduction of oxygen to water. Superoxide is produced from both Complexes I and III of the electron transport chain, and once in its anionic form, it is too strongly charged to readily cross the inner mitochondrial membrane. In Complex I (NADH dehydrogenase, also called NADH: ubiquinone oxidoreductase; EC 1.6.5.3) two electrons are removed from NADH and transferred to a lipid-soluble carrier, ubiquinone (Q). The reduced product, ubiquinol (QH_2) freely diffuses within the membrane, and Complex I translocates four protons (H^+) across the membrane, thus producing a proton gradient. Complex I, is one of the main sites at which premature electron leakage to oxygen occurs, thus being one of the main sites of production of harmful superoxide.

The pathway of electrons occurs as follows: NADH is oxidized to NAD^+, by reducing Flavin mononucleotide to $FMNH_2$ in a two-electron step. $FMNH_2$ is then oxidized in two one-electron steps, through a semiquinone intermediate. Each electron thus transfers from the $FMNH_2$ to a Fe-S cluster, from the Fe-S cluster to

ubiquinone (Q). Transfer of the first electron results in the free-radical (semiquinone) form of Q, and transfer of the second electron reduces the semiquinone form to the ubiquinol form, QH_2. During this process, four protons are translocated from the mitochondrial matrix to the intermembrane space.

In Complex III (cytochrome bc_1 complex; EC 1.10.2.2), the Q-cycle contributes to the proton gradient by an asymmetric absorption/release of protons. Two electrons are removed from QH_2 at the Q_O site and sequentially transferred to two molecules of cytochrome *c*, a water-soluble electron carrier located within the intermembrane space. The two other electrons sequentially pass across the protein to the Q_i site where the quinone part of ubiquinone is reduced to quinol. A proton gradient is formed by two quinol (4H+4e-) oxidations at the Q_o site to form one quinol (2H+2e-) at the Q_i site. In total six protons are translocated: two protons reduce quinone to quinol and four protons are released from two ubiquinol molecules. When electron transfer is reduced,by a high membrane potential or respiratory inhibitors such as antimycin A, Complex III may leak electrons to molecular oxygen, resulting in superoxide formation.

Recently, it has been demonstrated that Complex I-dependent superoxide is exclusively released into the matrix and that no detectable levels escape from intact mitochondria (Muller et al., 2004). This finding fits well with the proposed site of electron leak at Complex I, namely the iron–sulphur clusters of the (matrix-protruding) hydrophilic arm. In addition, experiments on Complex III show direct extra mitochondrial release of superoxide, but measurements of hydrogen peroxide production revealed that this could only account for <50% of the total electron leak even in mitochondria lacking Cu, Zn-SOD. It has been proposed that the remaining 50% of the electron leak must be due to superoxide released to the matrix. The hydroxyl radical, $OH^{\bullet}$, is the neutral form of the hydroxide ion. The hydroxyl radical has a high reactivity, making it a very dangerous radical with a very short *in vivo* half-life of approximately 10^{-9} s (Pastor et al., 2000). Thus when produced *in vivo* $OH^{\bullet}$ reacts close to its site of formation.

The redox state of the cell is largely linked to an iron (and copper) redox couple and is maintained within strict physiological limits. It has been suggested that iron regulation ensures that there is no free intracellular iron; however, *in vivo*, under stress conditions, an excess of superoxide releases “free iron” from iron-containing molecules. The release of iron by superoxide has been demonstrated for [4Fe–4S] cluster containing enzymes of the dehydratase-lyase family (Liochev and Fridovich, 1994). The released Fe^{2+} can participate in the Fenton reaction, generating highly reactive hydroxyl radical [Fe^{2+} (Mn^{+})+H_2O_2→Fe^{3+} (Mn^{++})+ $OH^{\bullet}$+OH^{-}, where Mn^{+} is any univalent metal cation].

Thus under stress conditions, $O_2^{\bullet-}$ acts as an oxidant of [4Fe–4S] cluster-containing enzymes and facilitates $OH^{\bullet}$ production from H_2O_2 by making Fe^{2+} available for the Fenton reaction (Valko et al., 2006; Leonard et al., 2004). The superoxide radical participates in the Haber–Weiss reaction ($O_2^{\bullet-}$ +H_2O_2→O_2 + $OH^{\bullet}$+OH^{-}) which combines a Fenton reaction and the reduction of Fe^{3+} by superoxide, yielding Fe^{2+} and oxygen (Fe^{3+} +$O^{2\ \bullet}$−→Fe^{2+} +O_2) (Liochev and Fridovich, 2002).The Fe–S cluster contains also iron responsive elements (IREs)-binding protein (IRE-BP). The iron-responsive element-binding proteins, also known as IRE-BP and IRBP and IRP, bind to IREs in the regulation of human iron metabolism. This Fe–S cluster has been implicated as the region of the protein that senses intracellular iron levels and accordingly modifies the ability of the IRE-BP to interact with IREs. IRE-BP produced in iron-replete cells has aconitase activity (Han et al., 2005).

In mammalian cells, oxidants are able to convert cytosolic aconitase into active IRE-BP, which increases the “free iron” concentration intracellularly both by decreasing the biosynthesis of ferritin and increasing biosynthesis of transferring receptors. The most realistic *in vivo* production of hydroxyl radical according to the Fenton reaction occurs when Mn^{+} is iron, copper, chromium, or cobalt. However, Rae and co-workers recently reported that the upper limit of so-called “free pools” of copper was far less than a single atom per cell (Rae et al., 1999). This finding casts serious doubt on the *in vivo* role of copper in Fenton-like generation of hydroxyl radical. Although Fenton chemistry is known to occur in vitro, its significance under physiological conditions is not clear, noting particularly the negligible availability of “free catalytic iron” due to its effective sequestration by the various metal-binding proteins (Kakhlon and Cabantchik, 2002).

However, organisms overloaded by iron (as in the conditions of hemochromatosis, b-thalassemia, hemodialysis), contain higher amounts of “free available iron” and this can have deleterious effects. “Free-iron” is transported into an intermediate, labile iron pool (LIP), which represents a steady state exchangeable, and readily chelatable iron compartment (Kakhlon and Cabantchik, 2002). Additional reactive radicals derived from oxygen that can be formed in living systems are peroxyl radicals ($ROO^{\bullet}$) as seen in Figure 2. The simplest peroxyl radical is $HOO^{\bullet}$, which is the protonated form (conjugate acid; *pKa* ~ 4.8) of superoxide ($O_2^{\bullet-}$) and is usually termed either hydroperoxyl radical or perhydroxyl radical.

Given this *pKa* value, only ~ 0.3% of any superoxide present in the cytosol of a typical cell is in the protonated form (De Grey, 2002). It has been demonstrated that hydroperoxyl radical initiates fatty acid peroxidation by two parallel pathways: fatty acid hydroperoxide (LOOH)-independent and LOOH-dependent (Aikens and Dix, 1991). The LOOH-dependent pathway of $HO_2^{\bullet}$ initiated fatty acid peroxidation may be relevant to mechanisms of lipid peroxidation initiation *in vivo*. Xanthine oxidase (XO,

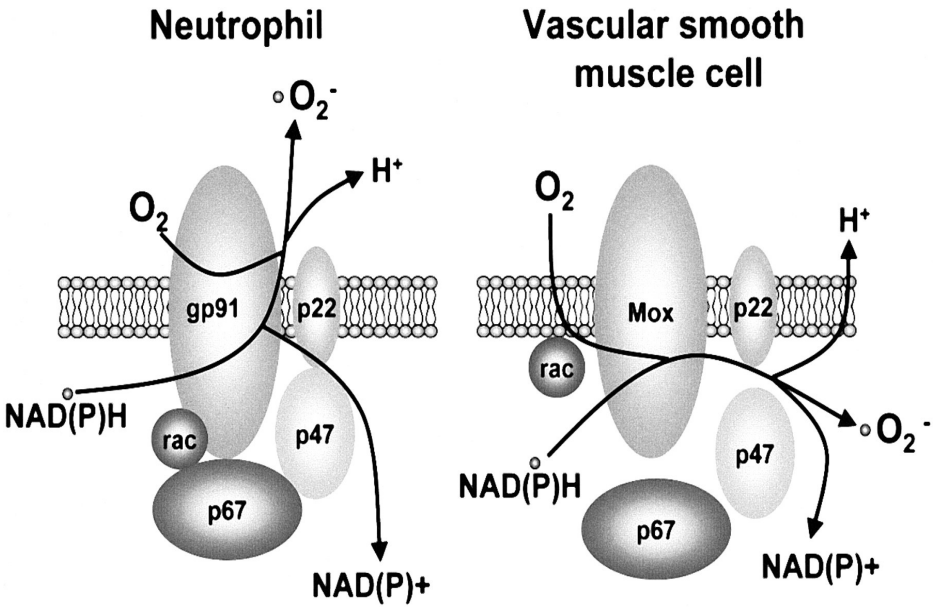
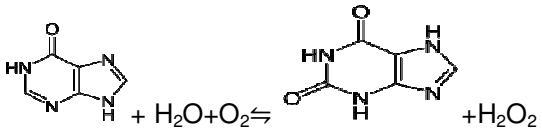
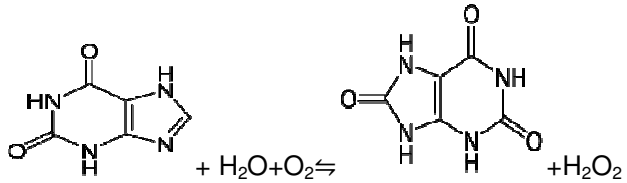


Figure 1. Structure of the NAD(P)H oxidase in neutrophils and vascular smooth muscles.

EC 1.1.3.22) and xanthine dehydrogenase (XD, EC 1.1.1.204) are interconvertible forms of the same enzyme, known as xanthine oxidoreductase (XOR). In purine catabolism, XOR catalyzes the oxidative hydroxylation of hypoxanthine to xanthine and subsequently of xanthine to uric acid. The following chemical reactions are catalyzed by xanthine oxidase:



Hypo-xanthine Xanthine



Uric acid acts as a potent antioxidant and free radical scavenger. XOR has, therefore, important functions as a cellular defense enzyme against oxidative stress. With both XO and XD forms, but particularly with the XO form, numerous ROS and RNS are synthesized (Vorbach et al., 2003). Thus, the synthesis of both an antioxidant (uric acid) and numerous free radicals (ROS and RNS) makes XOR an important protective regulator of the cellular redox potential. Xanthine oxidase can also act on certain other purines, pterins, and aldehydes. For example, it efficiently converts 1-methylxanthine (a metabolite of caffeine) to 1-methyluric acid, but has little activity on 3-methylxanthine. Peroxisomes are known to produce H_2O_2, but not $O_2^{\bullet-}$, under physiologic conditions (Valko et al., 2004).

Peroxisomes are major sites of oxygen consumption in the cell and participate in several metabolic functions that use oxygen. Oxygen consumption in the peroxisome leads to H_2O_2 production, which is then used to oxidize a variety of molecules. The organelle also contains catalase, which decomposes hydrogen peroxide and presumably prevents accumulation of this toxic compound. Thus, the peroxisome maintains a delicate balance with respect to the relative concentrations or activities of these enzymes to ensure the absence of net production of ROS. How the organelle maintains this equilibrium is unclear. When peroxisomes are damaged and their H_2O_2 consuming enzymes down regulated, H_2O_2 releases into the cytosol, which significantly contributes to oxidative stress. If a phagocytic cell such as the neutrophil is exposed to a stimulus, it has the ability of recognising the foreign particle and undergoing a series of reactions called the respiratory burst (DeCoursey and Ligeti, 2005).

Nicotine adenine dinucleotide phosphate (NAD(P)H) oxidase is best characterised in neutrophils, where its production of $O_2^{\bullet-}$ generates the respiratory burst necessary for bacterial destruction. The enzyme complex consists of two membrane-bound components, gp91phox and p22phox, which comprise cytochrome *b*558, the enzymatic centre of the complex. After activation, cytosolic components, involving p47phox, p67phox, p40phox and the small G coupled proteins, Rac and Rap1A, translocates to the membrane, to form the active enzyme complex shown in Figure 1. The nonphagocytic

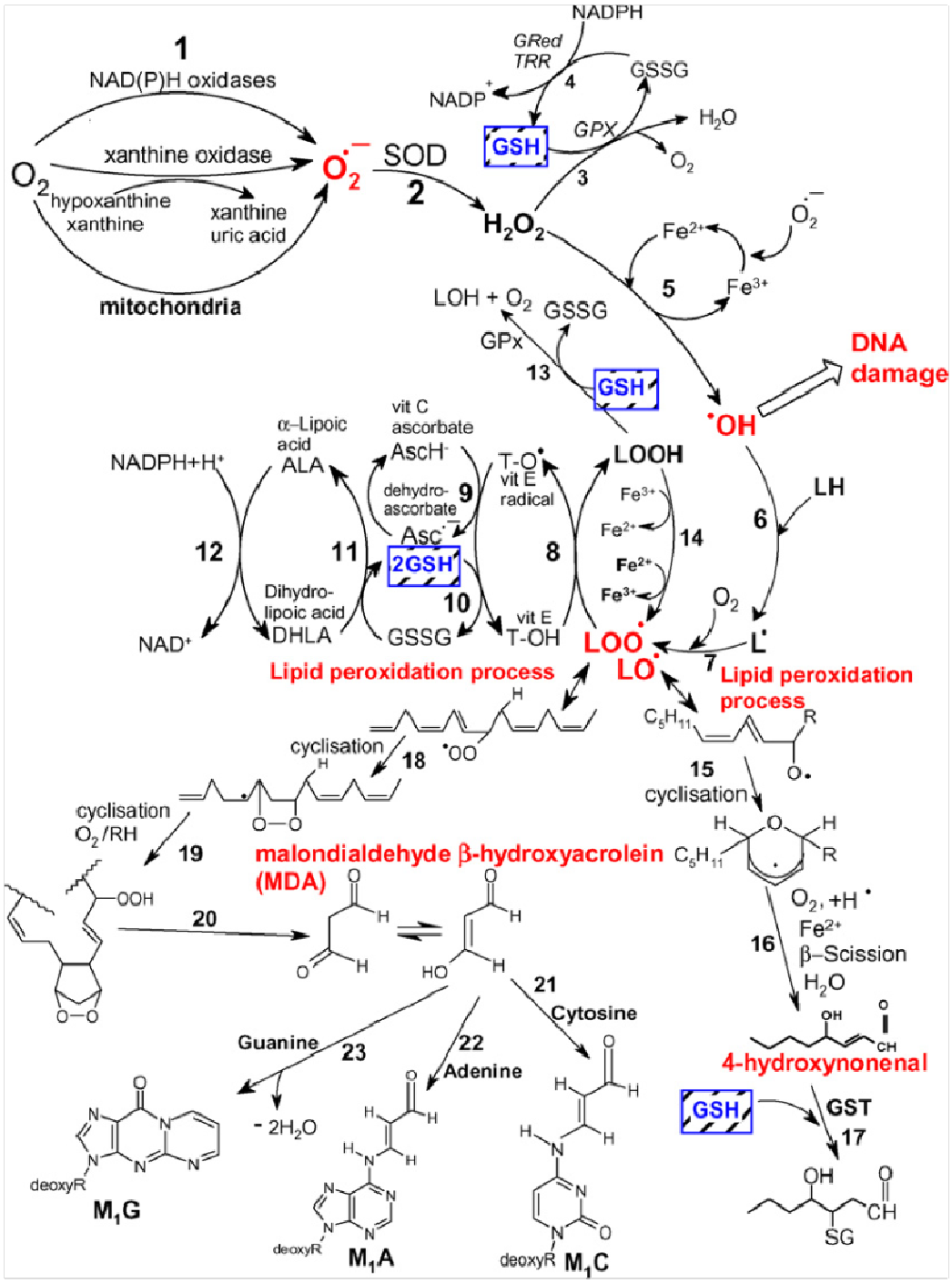


Figure 2. Pathways of ROS formation.

NAD(P)H oxidase, produce superoxide at a fraction (1–10%) of the levels produced in neutrophils and are thought to function in intracellular signaling pathways, as shown in Figure 1.

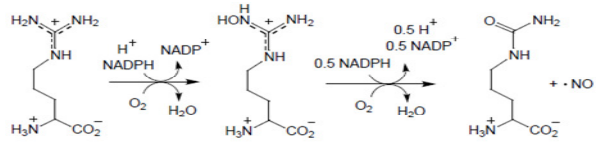
As can be seen in Figure 2, Pathways of ROS formation, the lipid peroxidation process and the role of glutathione (GSH) and other antioxidants (Vitamin E, Vitamin C, lipoic acid) in the management of oxidative stress, shows an unbalanced equation.

Reaction 1: The superoxide anion radical is formed by the process of reduction of molecular oxygen mediated by NAD(P)H oxidases and xanthine oxidase or non-

enzymatically by redox-reactive compounds such as the semi-ubiquinone compound of the mitochondrial electron transport chain.
Reaction 2: Superoxide radical is dismutated by the superoxide dismutase (SOD) to hydrogen peroxide.
Reaction 3: Hydrogen peroxide is most efficiently scavenged by the enzyme glutathione peroxidase (GPx) which requires GSH as the electron donor.
Reaction 4: The oxidised glutathione (GSSG) is reduced back to GSH by the enzyme glutathione reductase (Gred) which uses NADPH as the electron donor.
Reaction 5: Some transition metals (e.g. Fe^{2+}, Cu+ and others) can breakdown hydrogen peroxide to the reactive hydroxyl radical (Fenton reaction).
Reaction 6: The hydroxyl radical can abstract an electron from polyunsaturated fatty acid (LH) to give rise to a carbon-centered lipid radical (L•).
Reaction 7: The lipid radical (L•) can further interact with molecular oxygen to give a lipid peroxyl radical (LOO•). If the resulting lipid peroxyl radical LOO• is not reduced by antioxidants, the lipid peroxidation process occurs (reactions 18–23 and 15–17).
Reaction 8: The lipid peroxyl radical (LOO•) is reduced within the membrane by the reduced form of Vitamin E (T-OH) resulting in the formation of a lipid hydroperoxide and a radical of Vitamin E (T-O•).
Reaction 9: The regeneration of Vitamin E by Vitamin C: the Vitamin E radical (T-O•) is reduced back to Vitamin E (T-OH) by ascorbic acid (the physiological form of ascorbate is ascorbate monoanion, AscH–) leaving behind the ascorbyl radical (Asc•–).
Reaction 10: The regeneration of Vitamin E by GSH: the oxidised Vitamin E radical (T-O•) is reduced by GSH.
Reaction 11: The oxidised glutathione (GSSG) and the ascorbyl radical (Asc•–) are reduced back to GSH and ascorbate monoanion, AscH–, respectively, by the dihydrolipoic acid (DHLA) which is itself converted to _-lipoic acid (ALA).
Reaction 12: The regeneration of DHLA from ALA using NADPH.
Reaction 13: Lipid hydroperoxides are reduced to alcohols and dioxygen by GPx using GSH as the electron donor.
Lipid peroxidation process: Reaction 14: Lipid hydroperoxides can react fast with Fe^{2+} to form lipid alkoxyl radicals (LO•), or much slower with Fe^{3+} to form lipid peroxyl radicals (LOO•).
Reaction 15: Lipid alkoxyl radical (LO•) derived for example from arachidonic acid undergoes cyclisation reaction to form a six-membered ring hydroperoxide.
Reaction 16: Six-membered ring hydroperoxide undergoes further reactions (involving_-scission) to from 4-hydroxy-nonenal.
Reaction 17: 4-hydroxynonenal is rendered into an innocuous glutathiyl adduct (GST, glutathione S-transferase).
Reaction 18: A peroxyl radical located in the internal position of the fatty acid can react by cyclisation to produce a cyclic peroxide adjacent to a carbon-centered radical.
Reaction 19: This radical can then either be reduced to form a hydroperoxide (reaction not shown) or it can undergo a second cyclisation to form bicyclic peroxide which after coupling to dioxygen and reduction, yields a molecule structurally analogous to the endoperoxide.
Reaction 20: Formed compound is an intermediate product for the production of malondialdehyde. Reactions 21, 22, 23: Malondialdehyde can react with DNA bases Cytosine, Adenine, and Guanine to form adducts M1C, M1A and M1G, respectively.

Reactive nitrogen species (RNS)

$NO^{\bullet}$ is a small molecule that contains one unpaired electron on the antibonding 2π* y orbital and is therefore, a radical. $NO^{\bullet}$ is generated in biological tissues by specific nitric oxide syntheses (NOS's), which metabolises arginine to citrulline with the formation of $NO^{\bullet}$ via a five electron oxidative reaction (Ghafourifar and Cadenas, 2005).



L-Arginine N-Hydroxyarginine L-Citrulline

Nitric oxide ($NO^{\bullet}$) is an abundant reactive radical that acts as an important oxidative biological signaling molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, defense mechanisms, smooth muscle relaxation and immune regulation (Bergendi et al., 1999). Due to its extraordinary properties, $NO^{\bullet}$ was acclaimed as the "molecule of the year" in Science Magazine (Koshland, 1992). $NO^{\bullet}$ has a half-life of only a few seconds in an aqueous environment. $NO^{\bullet}$ has greater stability in an environment with a lower oxygen concentration (half life >15 s). However, since it is soluble in both aqueous and lipid media, it readily diffuses through the cytoplasm and plasma membranes (Chiueh, 1999).

$NO^{\bullet}$ has effects on neuronal transmission as well as on synaptic plasticity in the central nervous system. In the extracellular milieu, NO• reacts with oxygen and water to form nitrate and nitrite anions. Overproduction of reactive nitrogen species is called nitrosative stress (Klatt and Lamas, 2000; Ridnour et al., 2004). This may occur when the generation of reactive nitrogen species 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 superoxide anion and nitric oxide during

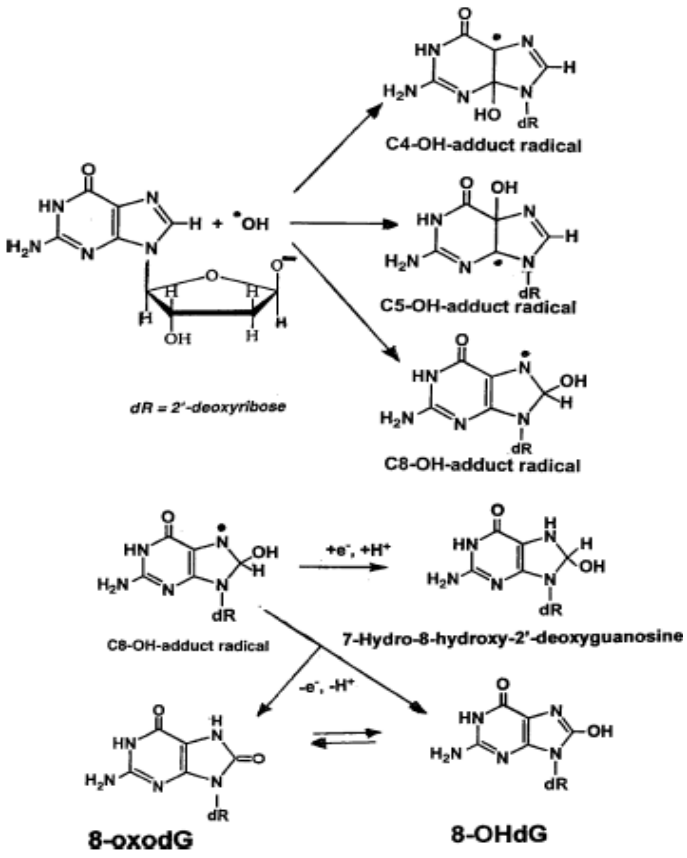


Figure 3. Reaction of 2_deoxyguanosine with hydroxyl radicals

the oxidative burst triggered during inflammatory processes. Under these conditions, nitric oxide and the superoxide anion may react together to produce significant amounts of a much more oxidatively active molecule, peroxynitrite anion ($ONOO^-$), which is a potent oxidising agent that can cause DNA fragmentation and lipid oxidation (Carr et al., 2000):

$$NO\bullet + O_2^{\bullet -} \rightarrow ONOO^- \quad (1)$$

Reaction (1) has one of the highest rate constants known for reactions of NO•, 7.0 × 109 $M^{-1}S^{-1}$. Thus NO• toxicity is predominantly linked to its ability to combine with superoxide anions. Nitric oxide readily binds certain transition metal ions; in fact many physiological effects of NO• are exerted as a result of its initial binding to Fe^{2+}-Haem groups in the enzyme soluble guanylate cyclase (sGC) (Archer, 1993).

$$Fe^{2+}\{sGC\} + NO^{\bullet} \rightarrow Fe^{2+}\{sGC\}–NO \quad (2)$$

The product is represented here as $\{Fe^{2+}–NO^{\bullet}\}$, however, $\{Fe^{3+}–NO^{-}\}$ is also commonly seen. The convention $\{FeNO\}^7$, where the superscript is the sum of the metal *d* electron count (here 6 or 5) and the occupancy of the relevant NO π orbital (here 1 or 2), is often employed to avoid specific assignment of oxidation states.

Oxidative damage to DNA, lipids and proteins

At high concentrations, ROS can be important mediators of damage to cell structures, nucleic acids, lipids and proteins (Valko et al., 2006). The hydroxyl radical is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone (Halliwell and Gutteridge, 1999). The most extensively studied DNA lesion is the formation of 8-OH-G (8-hydroxy-2-deoxyguanosine) as shown in Figure 3. Figure 3 shows reaction of 2_deoxyguanosine with hydroxyl radicals, radical adducts followed by reduction to 7-hydro-8-hydroxy-2_deoxyguanosine, and by oxidation to 8-hydroxy-2-deoxyguanosine (8-OHdG) or its tautomer 8-oxo-7-hydro-2_deoxyguanosine (8-oxodG).

Permanent modification of genetic material resulting from “oxidative damage” incidents represents the first step involved in mutagenesis, carcinogenesis, and

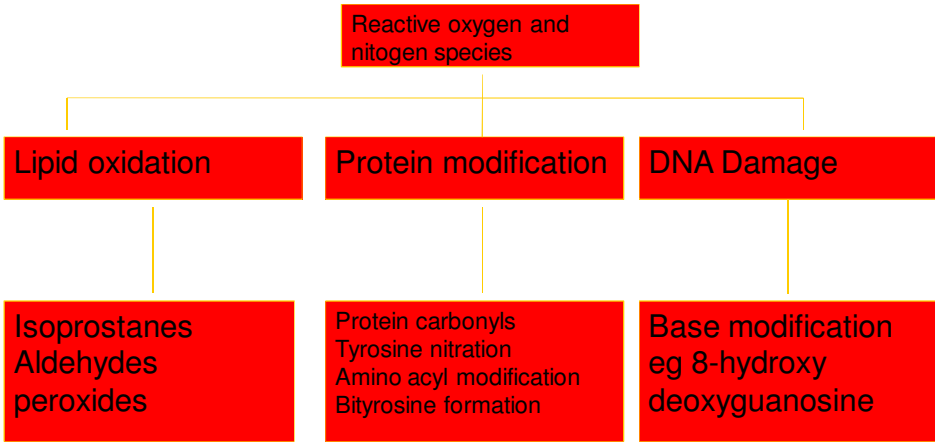


Figure 4. Various biomarkers of oxidative damage (adapted from Rice Evans, 1999).

ageing. It is known that metal-induced generation of ROS results in an attack not only on DNA, but also on other cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation (Siems et al., 1995).

Once formed, peroxyl radicals ($ROO^{\bullet}$) can be rearranged via a cyclisation reaction to endoperoxides (precursors of malondialdehyde) with the final product of the peroxidation process being malondialdehyde (MDA) (Wang et al., 1996) as shown in Figure 2. The major aldehyde product of lipid peroxidation other than malondialdehyde is 4-hydroxy- 2-nonenal (HNE). MDA is mutagenic in bacterial and mammalian cells and carcinogenic in rats. Hydroxynonenal is weakly mutagenic but appears to be the major toxic product of lipid peroxidation. Mechanisms involved in the oxidation of proteins by ROS were elucidated by studies in which amino acids, simple peptides and proteins were exposed to ionizing radiations under conditions where hydroxyl radicals or a mixture of hydroxyl/superoxide radicals are formed (Stadtman, 2004). The side chains of all amino acid residues of proteins, in particular cysteine and methionine residues of proteins are susceptible to oxidation by the action of ROS/RNS (Stadtman, 2004; Dalle-Donne et al., 2005).

Oxidation of cysteine residues may lead to the reversible formation of mixed disulphide between protein thiol groups (–SH) and low molecular weight thiols, in particular GSH (S-glutathiolation). The concentration of carbonyl groups, generated by many different mechanisms is a good measure of ROS-mediated protein oxidation. A number of highly sensitive methods have been developed for the assay of protein carbonyl groups (Dalle-Donne et al., 2005).

Advanced glycation end products (AGEs) is a class of complex products. They are the results of a reaction between carbohydrates and free amino group of proteins. The intermediate products are known, variously, as Amadori, Schiff Base and Maillard products, named after the researchers who first described those (Dalle-Donne et al., 2005). Most of the AGEs are very unstable, reactive compounds and the end products are difficult to be completely analyzed. The brown colour of the AGEs is probably related to the name of melanoidins initially proposed by Maillard (1912), and well known from food chemistry. The best chemically characterized AGEs compounds found in human are pentosidine and carboxyl methyl lysine (CML). Various biomarkers of oxidative damage are shown in Figure 4.

Antioxidants

Exposure to free radicals from a variety of sources has led organisms to develop a series of defense mechanisms (Cadenas, 1997). Defense mechanisms against free radical-induced oxidative stress involve: (i) preventative mechanisms, (ii) repair mechanisms, (iii) physical defences, and (iv) antioxidant defences. Enzymatic antioxidant defences include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT). Non-enzymatic antioxidants are represented by ascorbic acid (Vitamin C), tocopherol (Vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants.

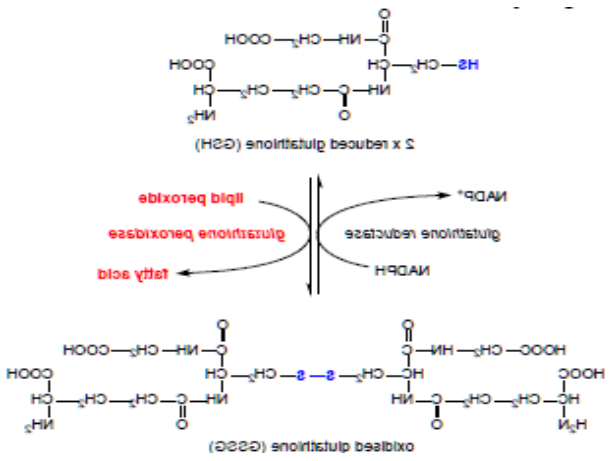
Under normal conditions, there is a balance between both the activities and the intracellular levels of these antioxidants (Liu, 2004). This balance is essential for the survival of organisms and their health. Various pathways for the management of oxidative stress by GSH and other antioxidants are shown in Figure 2. Here, we briefly describe the role of major thiol antioxidant and redox buffer of the cell, the tripeptide, glutathione (GSH) (Masella et al., 2005).

The oxidised form of glutathione is GSSG, glutathione disulphide. Glutathione is highly abundant in the cytosol

(1–11 mM), nuclei (3–15 mM), and mitochondria (5–11 mM) and is the major soluble antioxidant in these cell compartments. Because GSH is synthesized in the cytosol by the sequential action of glutamate-cysteine ligase and glutathione synthetase, its mitochondrial presence requires inner membrane transport. Two mitochondrial electroneutral antiport carrier proteins have been shown to have the capacity to transport GSH, the dicarboxylate carrier protein and the 2-oxoglutarate carrier protein.

Recently, it has been shown that externally added GSH is readily taken up by Mitochondria, despite the ~8mM GSH present in the mitochondrial matrix (Shen et al., 2005). It therefore appears that GSH is taken up against a concentration gradient. GSH in the nucleus maintains the redox state of critical protein sulphydryls that are necessary for DNA repair and expression. Oxidised glutathione is accumulated inside the cells and the ratio of GSH/GSSG is a good measure of oxidative stress of an organism (Jones et al., 2000). Too high a concentration of GSSG may damage many enzymes oxidatively. The main protective roles of glutathione against oxidative stress are according to (Masella et al., 2005): (i) glutathione is a cofactor of several detoxifying enzymes against oxidative stress, e.g. glutathione peroxidase (GPx), glutathionetransferase and others; (ii) GSH participates in amino acid transport through the plasma membrane; (iii) GSH scavenges hydroxyl radical and singlet oxygen directly, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of glutathionperoxidase; (iv) glutathione is able to regenerate the most important antioxidants, Vitamins C and E, back to their active forms; glutathione can reduce the tocopherol radical of Vitamin E directly, or indirectly, via reduction of semidehydroascorbate to ascorbate (Figure 2).

The capacity of glutathione to regenerate the most important antioxidants is linked with the redox state of the glutathione disulphide-glutathione couple (GSSG/2GSH) (Pastore et al., 2003). The basic mechanism of action of GSH is illustrated as follows:



The various roles of enzymatic antioxidants (SOD, Catalase, and glutathione peroxidase) and non-enzymatic antioxidants (Vitamin C, Vitamin E, carotenoids, lipoic acid and others) in the protection against oxidative stress can be found in a numerous reviews and original papers (White et al., 1997) as shown in Figure 2.

ROS and mechanisms of maintenance of "redox homeostasis"

Free radicals and reactive diamagnetic species derived from radicals operate at low, but measurable concentrations in the cells. Their "steady state" concentrations are determined by the balance between their rates of production and their rates of removal by various antioxidants. Thus each cell is characterised by a particular concentration of electrons (redox state) stored in many cellular constituents and the redox state of a cell and its oscillation determines cellular functioning (Schafer and Buettner, 2001).

In recent years the term "redox state" has not only been used to describe the state of a redox pair, e.g. GSSG/2GSH, Asc•–/AcsH– and others, but also to describe more generally the redox environment of a cell (Butler, 2000; Schafer and Buettner, 2001). The redox state of a cell is kept within a narrow range under normal conditions, similar to the manner in which a biological system regulates its pH. Under pathological conditions, the redox state can be altered to lower or higher values. A 30mV change in the redox state means a 10-fold change in the ratio between reductant and oxidant species (Schafer and Buettner, 2001). The intracellular "redox homeostasis" or "redox buffering" capacity is substantiated primarily by GSH and thioredoxin (TRX). The glutathione (2GSH/GSSG couple) represents the major cellular redox buffer and therefore is a representative indicator for the redox environment of the cell (Droge, 2002; Schafer and Buettner, 2001).

Under enhanced oxidative stress conditions, GSSG content increases, this in turn increases the content of protein mixed disulphide. A significant number of proteins involved in signaling that have critical thiols, such as receptors, protein kinases and some transcription factors can be altered in their function by formation of mixed disulphides (Thannickal and Fanburg, 2000). In this regard, GSSG appears to act as a non-specific signalling molecule. The high ratios of reduced to oxidised GSH and TRX are maintained by the activity of GSH reductase and TRX reductase, respectively. Both of these "redox buffering" thiol systems counteract intracellular oxidative stress; in addition to antioxidant functioning in the cell, GSH and TRX are involved in cell signaling process (Droge, 2002; Thannickal and Fanburg, 2002).

In addition to GSH and TRX, there are other relatively low molecular weight antioxidants, that when present at

high concentration, can significantly contribute to overall ROS scavenging activity (McEligot et al., 2005). These include various free amino acids, peptides, and proteins. Oxidised proteins are substrates for proteolytic digestion and contribute to maintenance of redox homeostasis in the cell (Dr¨oge, 2002). Oxidative modifications of proteins increase their susceptibility to proteolytic attack; proteolytic degradation is executed mainly by proteasomes. Proteolysis was estimated to increase more than 10-times after exposure to superoxide radical or hydrogen peroxide. It should be noted that proteins significantly vary in their susceptibility to oxidative damage. For example, intact proteins are less sensitive to oxidation than misfolded proteins.

The term redox signalling is used to describe a regulatory process in which the signal is delivered through redox reactions. Redox signalling requires that the steady state of "redox balance" is disturbed either by an increase in ROS formation or a decrease in the activity of antioxidant system(s). The regulated increase in free radicals (ROS/RNS) leads to a temporary imbalance that represents the physiological basis for redox regulation. Thus, physiological demonstration of redox regulation involves a temporary shift of the intracellular redox state toward more oxidising conditions.

Signalling mechanisms that respond to changes in the thiol/disulphide redox state involve: (i) transcription factors AP-1 and NF-κB; the transcription factor NF-κB was originally studied in cells of the immune system wherein it regulates cell survival (Baeuerle and Baltimore, 1996), it is widely expressed in the nervous system and exists in neurons in both an inducible and a constitutively active form (Kaltschmidt et al., 1993). NF-κB resides in the cytoplasm in an inactive form consisting of three subunits: p65 and p50 and an inhibitory subunit called IκB (Siebenlist et al*.,* 1994). When IκB is bound to p50/p65, it is inactive; signals that activate NF-κB causes dissociation of IκB releasing p50/p65, which then translocates to the nucleus and binds to specific κB DNA consensus sequences in the enhancer region of a variety of κB-responsive genes (Mattson et al., 2000). In neurons, NF-κB is activated by various intercellular signals, including cytokines, neurotrophic factors, and neurotransmitters (Carter et al., 1996). (ii) bacterial OxyR; (iii) protein tyrosine phosphatases; (iv) Src family kinases; (v) JNK and p38 MAPK signaling pathways; (vi) insulin receptor kinase activity, and others (Droge, 2002; Aslund et al., 1999).

Under pathological conditions, however, abnormally large concentrations of ROS/RNS may lead to permanent changes in signal transduction and gene expression, typical for disease states. The process of redox signaling is adopted by various organisms including bacteria to induce protective responses against oxidative stress and to restore the original state of "redox homeostasis" after temporary exposure to ROS/RNS. For example, the production of NO• is the subject of direct feedback inhibition of NOS by NO•. Prokaryotes have several different signalling pathways for responding to ROS or to alterations in the intracellular redox state. Studies on *Escherichia coli* explored that low levels of ROS activate expression of several gene products involved in antioxidant defense including Mn- SOD, catalase, glutathione reductase, and others.

Several proteins that are synthesised in *Escherichia coli* after exposure to hydrogen peroxide are under the control of the OxyR locus. The OxyR protein controls protective responses against lethal doses of hydrogen peroxide or against killing by heat (Aslund et al., 1999). Hydrogen peroxide or an oxidative shift in the intracellular thiol/disulphide redox state converts the reduced form of OxyR (containing –SH groups) into its oxidised and regulatory active form containing –S–S– groups. The formation of disulphide bonds can be reversed by glutaredoxin and by thioredoxin. One of the best studied models of redox regulation in mammalian cells is the redox control of heme oxygenase-1 (HO-1) (Keyse and Tyrrell, 1989).

HO-1 induction in skin fibroblasts may serve as an inducible defence pathway to remove heme liberated by oxidants. The HO-1 protein and mRNA are strongly induced by ROS, UVA irradiation and various stressors; thus the inducibility of HO-1 mRNA in many tissues and various mammalian species has rendered HO-1 mRNA a useful marker for cellular oxidative stress at the mRNA level. As mentioned above, the cell cycle is characterized by fluctuations in the redox environment of a cell, mediated, in particular by intracellular changes in concentration of glutathione (Kern and Kehrer, 2005; Schafer and Buettner, 2001).

GSH has been shown to play a role in the rescue of cells from apoptosis; depletion of GSH, which renders the cellular environment more oxidising, was concomitant with the onset of apoptosis. Generally, a more reducing environment (maintained by elevated levels of glutathione and thioredoxin) of the cell stimulates proliferation and a slight shift towards a mildly oxidizing environment initiates cell differentiation. A further shift towards a more oxidising environment in the cell leads to apoptosis and necrosis. While apoptosis is induced by moderate oxidising stimuli, necrosis is induced by an intense oxidising effect (Evens, 2004; Voehringer et al., 2000). From the above discussion it is clear that the redox environment is the critical determinant for the trigger of apoptosis. Recent studies indicate that a knowledge of the mechanisms by which TRX, GSH, and Ref-1 maintain the intracellular "redox buffering" capacity can conveniently be used in the development of targeted cancer-preventive and therapeutic drugs (Evens, 2004).

OXIDATIVE STRESS AND DAMAGE

What happens if the balance between ROS and antioxi-

dants is upset? Having too many ROS in relation to the available antioxidants is said to be a state of oxidative stress. Sies (1991), defined this term as a disturbance in the prooxidant-antioxidant balance in favor of the former, leading to potential damage. Such damage is often called oxidative damage, which has been defined as the biomolecular damage caused by attack of reactive species upon the constituents of living organisms (Halliwell and Whiteman, 2004).

Increased oxidative damage can result not only from more oxidative stress, but also from failure to repair or replace damaged biomolecules. Oxidative stress can result from decreases in antioxidant levels, e.g. mutations decreasing the levels of MnSOD; depletions of dietary antioxidants and other essential dietary constituents (e.g. copper, iron, zinc, and magnesium) can also cause it. For example, children with the protein deficiency disease,Kwashiorkor, suffer oxidative stress, involving low GSH levels (lack of sulfur-containing amino acids in the diet) and iron overload (inability to make enough transferrin.

Oxidative stress can also be due to increased ROS production for instance, by exposure to elevated O2; the presence of toxins that produce ROS (e.g. paraquat), or excessive activation of natural systems producing ROS, e.g. inappropriate activation of phagocytes (Halliwell and Gutteridge, 2006; Halliwell, 2000). What do cells do when under oxidative stress? It depends on the cell and the level of stress applied (Figure 4). Usually intracellular free Ca^{2+} levels rise; so do levels of iron catalytic for free radical reactions shown in Figure 4.

Several cell types respond to mild oxidative stress by proliferating, which can be good in wound healing but bad if it leads to tissue fibrosis (Cave et al., 2005). Cells may adapt to the stress by up-regulation of defense and/or repair systems. This may completely protect against damage, to some extent but not completely, or sometimes overprotect; the cells are then resistant to higher levels of oxidative stress imposed subsequently. Adaptation need not always involve increases in antioxidants; there can be decreases in ROS-producing systems, increases in other protective mechanisms (such as chaperones), or changes in oxidative damage targets (e.g. *E. coli* under oxidative stress, can replace a fumarase enzyme sensitive to inactivation by O_2 with one that resists O_2. Moderate oxidative stress usually halts the cell cycle, or can drive cells into senescence; the cell survives but can no longer divide. Severe oxidative damage, especially to DNA, may trigger death by apoptosis, necrosis, or mechanisms with features of both. Indeed, ROS act as triggers of apoptosis, and as participants in apoptosis induced by other mechanisms, in both plants and animals.

Figure 5, shows how cells respond to oxidative stress (Halliwell and Gutteridge, 2006). Stimulation of proliferation by low levels of RS (Reactive species) is associated with increased net phosphorylation of multiple proteins, often because of RS inactivate protein phosphatase enzymes (Kang et al., 2005) and sometimes because of increased protein kinase activity (Storz at al*.,* 2004). The cell is generally a reducing environment, especially the mitochondria (GSH/GSSG>100) and cytosol (GSH/GSSG>100), but less so in the endoplasmic reticulum lumen (GSH/GSSG≈3), since a more oxidizing environment is required for optimal protein folding and disulfide bridge formation, ER (endoplasmic reticulum); HO-1, (haem oxygenase 1).

The following notes relate to the numbers in Figure 5: [1]'Reactive species' is a collective term that includes reactive oxygen, nitrogen, and halogen and sulfur species. For example, the term 'reactive oxygen species' (ROS) includes both oxygen-centered radicals (e.g. hydroxyl, superoxide, peroxyl) and certain non-radicals that are oxidizing agents and/or readily converted into radicals (e.g. peroxynitrite, hypochlorous acid). Similarly, reactive nitrogen species (RNS) includes the radicals' nitric oxide, and nitrogen dioxide as well as non-radicals such as nitryl chloride and nitroxyl anion. Many (but not all) RS are powerful oxidizing agents, capable of damaging DNA and other biomolecules. For a full description of RS and discussion of terminology, please see Halliwell and Gutteridge (2006).[2]Oxidative stress is a serious imbalance between RS levels and antioxidants, leading to potential damage. [3]Oxidative damage is the biomolecular damage caused by direct attack of RS. Its cellular levels are controlled by the balance between rate of damage and rate of repair or replacement of damaged biomolecules. [4]Caspase activity can also be modulated by changes in intracellular pH caused by RS (Parveiz and Clement, 2004; Akram et al*.,* 2006*).*

ANTIOXIDANTS AND THE PREVENTION OF CANCER

Mechanisms of action

Cancer is the end point of a multistep process involving a sequence of events that occur over a period of years or even decades. DNA damage is considered to be one of the most important contributors to cancer. Much of this damage is oxidative in nature. A marker of mutagenic DNA damage would be useful in the estimation of cancer risk of various populations and in monitoring the effects of chemoprevention. Much of this damage is oxidative in nature. It has been estimated that a typical human cell experiences about 10000 oxidative "hits" to its DNA each day. DNA repair enzymes remove most of this damage, but not all of it. Oxidative lesions to DNA accumulate with age, and so does the risk of cancer. If a cell containing damaged DNA divides before its DNA can be repaired, the result is a permanent genetic alteration, the first step in carcinogenesis.

Cells that divide rapidly are more susceptible to carcin-

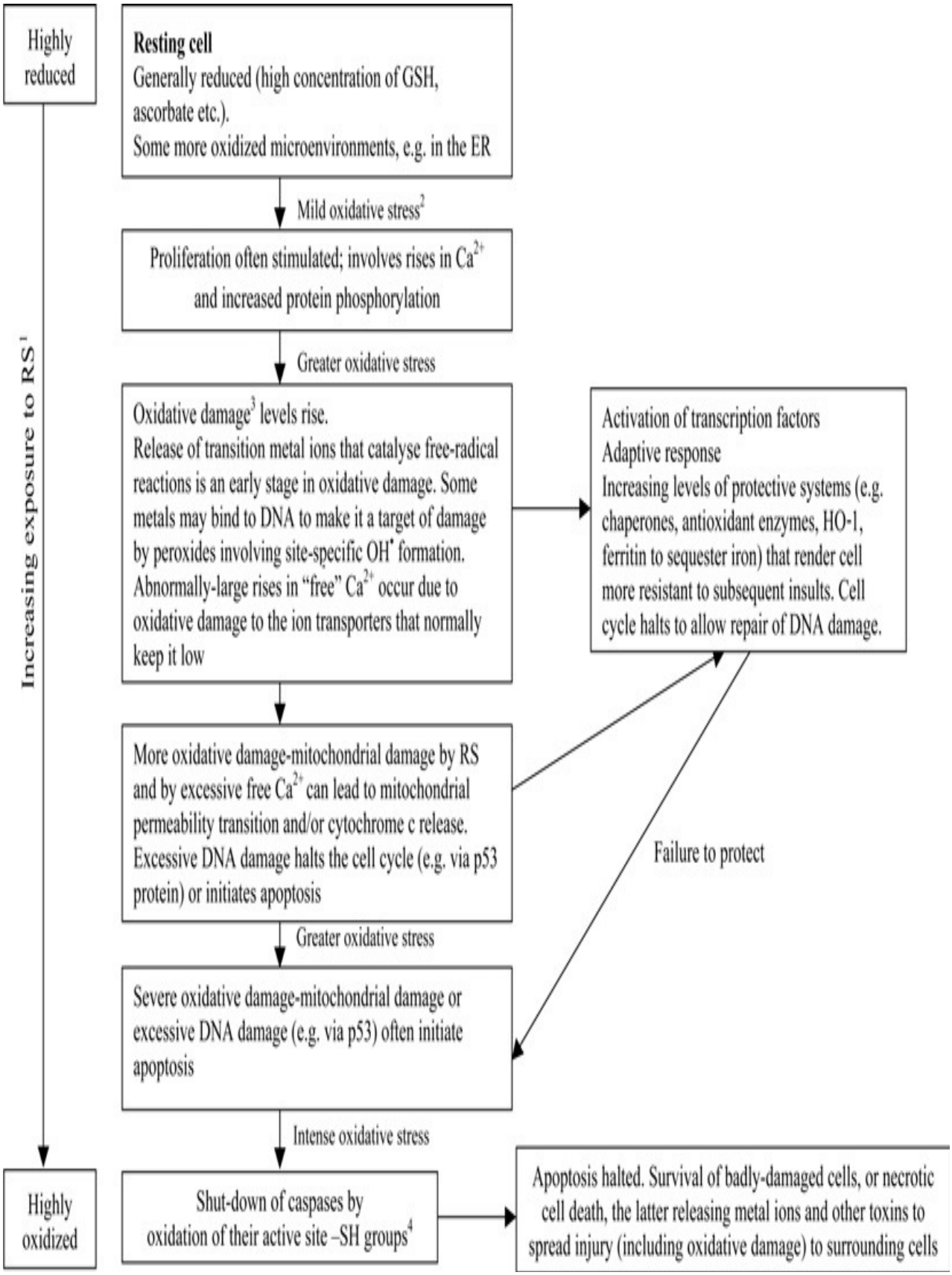


Figure 5. Cells respond to oxidative stress.

ogenesis than slowly dividing cells because there is less opportunity for DNA repair before cell division. Oxidants and antioxidants may also play a role in the later stages of cancer development. There is increasing evidence that oxidative processes contribute to the promotion stage of carcinogenesis, although the mechanisms for this are not well understood. Antioxidants may be able to cause the regression of premalignant lesions or inhibit their development into cancer.

Preliminary studies have indicated that some antioxidants, particularly ß-carotene, may be of benefit in the treatment of precancerous conditions such as oral leukoplakia (which may be a precursor of oral cancer). Some antioxidant nutrients may protect against cancer through mechanisms other than their antioxidant properties. For example, carotenoids may both enhance immune function and increase gap junctional communication (a type of interaction between cells that inhibits cell proliferation); both of these actions may be relevant to cancer prevention.

Tissue levels of antioxidants

Some epidemiologic studies have used biomarkers — physiological indicators of antioxidant status — rather than dietary data as measures of exposure. The most commonly used short-term biomarker is the concentration of an antioxidant in blood plasma. The findings of a major Chinese study support the concept that antioxidants may be protective against cancer. This study was conducted in 65 Chinese counties with an extraordinary diversity of disease patterns. Blood samples were collected from 100 randomly selected residents of each county; samples from members of the same community were then pooled for analysis. There was a consistent inverse correlation between cancer death rates in the 65 counties and blood levels of ß-carotene, vitamin C and selenium. For a wide range of cancers, associations between low death rates and high vitamin C levels were stronger than those for any other nutrient. High ß-carotene levels were associated with a reduced risk of stomach cancer. The EURAMIC study (European Community Multicentre Study on Antioxidants, Myocardial Infarction and Breast Cancer), now in progress, is using adipose tissue concentrations of vitamin E and ß-carotene and concentrations of selenium as biomarkers of antioxidant status. These biomarkers were chosen because they are believed to be an integrated measure of exposure over months rather than days. Epidemiologic studies in six European locations (Berlin, Germany; Coleraine, United Kingdom; Granada, Spain; Malaga, Spain; Zeist, The Netherlands; and Zürich, Switzerland) will compare levels of these biomarkers in breast cancer patients with those in otherwise similar women without the disease who live in the same areas.

Effects of individual carotenoids

In a few recent studies, epidemiologists have attempted to distinguish effects of specific carotenoids, rather than focusing on total carotenoids or ß-carotene. The results of these studies have suggested that several different carotenoids may be associated with reduced cancer risks. For example, in a study conducted in Hawaii, high dietary intakes of ß-carotene, α-carotene and lutein were each associated with reduced risks of lung cancer. Another study in the United States found that blood levels of total carotenoids, α- carotene, ß-carotene, cryptoxanthin and lycopene, but not lutein, were lower among women who later developed cervical cancer than among those who remained healthy. In the same U.S. population, blood levels of five major carotenoids were all found to be lower among individuals who later developed oral cancer than among controls.

Vitamin E

The evidence linking vitamin E and cancer risk is less extensive than that for vitamin C and carotenoids. Until recently, a lack of reliable information on the vitamin E content in foods has impeded epidemiologic studies of dietary vitamin E intake. Instead, most researchers have used blood vitamin E levels as a biomarker of vitamin E nutriture. The findings of these blood studies have been inconsistent; some have shown an inverse association between vitamin E levels and cancer risk, whereas others have found no association.

A large multicentre case-control study conducted by the U.S. National Cancer Institute, associated the use of vitamin E supplements with a 50% reduction in oral cancer risk. Dietary vitamin E and multivitamins had no significant effect. Dietary vitamin E intake in the United States is generally less than 15 international units (IU)/day (11 mg α-tocopherol equivalents [ATE]/day); multivitamins provide 30 IU/day (22 mg ATE/day); and most single-entity supplements sold in the United States contain at least 100 IU (74 mg ATE). This suggests the possibility that vitamin E may show a "plateau effect", meaning that below a certain critical dose it may not have a detectable inverse association with cancer risk. The plateau dose is likely to be greater than that which can be obtained through diet alone.

It is also possible, however, that the relationship between vitamin E supplementation and oral cancer seen in this study may not have been causal; people who chose to use supplements may have differed from nonusers in other ways that might affect oral cancer risk. At present, the use of supplements is an individual decision, and consumers receive little guidance about appropriate types and doses.

In general, individuals who choose to take supplements tend to make other efforts to improve their health as well,

reflecting a generally health conscious lifestyle.

Intervention trials

Several clinical intervention trials designed to evaluate the effect of antioxidant supplementation on cancer risk are now in progress as shown in Table 1. Studies of this type are the best way to establish definitively the effects of a specific substance. However, cancer prevention intervention trials must involve large numbers of subjects and lengthy periods of treatment. Therefore, intervention trials are usually undertaken only when substantial evidence from other types of investigations suggests that an effect is likely to be demonstrated.

The Linxian trials were designed to determine the effect of nutrient supplementation on cancer risk in the general population. Participants in the trial either received various combinations of nutrient supplements or received placebos daily for 5 years. One of the supplements (a ß-carotene/vitamin E/selenium combination) was found to reduce stomach cancer deaths by 21%, total cancer deaths by 13% and total mortality by 9%. Thus, one or more of these antioxidants appear to be protective against cancer in the population studied. However, the results of this trial may not be directly applicable to Western populations, where both dietary habits and disease patterns are drastically different from those of this nutritionally deprived Chinese community. The other Linxian trial evaluated the effects of multivitamin and ß-carotene supplementation on oesophageal cancer risk in individuals who already had oesophageal dysplasia, a precancerous condition. More than 3.000 subjects received either supplements or placebos daily for 6 years. The results of the study were negative; supplementation did not reduce oesophageal cancer risk.

The Finnish trial, conducted by the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, evaluated the effect of vitamin E and ß-carotene on the incidence of lung cancer and other cancers in 29,000 men who had smoked for an average of 35.9 years. The men were randomly assigned to one of four regimens: ß-carotene (20 mg/day), vitamin E (50 mg/day as dl-α-tocopheryl acetate), both supplements together at the same levels, or an inactive placebo. Follow-up continued for 5 to 8 years, with lung cancer as the primary endpoint. Subjects taking ß-carotene either alone or with vitamin E were compared with subjects not taking ß-carotene. Contrary to expectations, the men who were taking ß-carotene showed a statistically significant 18% increase in lung cancer incidence. There was no evidence of an interaction between vitamin E and ß carotene with respect to lung cancer. ß-Carotene had some effect on the incidence of cancers other than lung cancer. The unexpected results of the Finnish trial should receive serious consideration, but the findings of a single study must always be placed in appropriate perspective. More studies are required.

Another cancer prevention trial, completed in 1994 in the United States, failed to show that a 4-year period of antioxidant treatment could prevent the occurrence of new colorectal adenomas in patients with a history of past adenomas. The 751 patients participating in this trial were randomly assigned to receive daily either ß-carotene (25 mg), vitamin E (400 mg) plus vitamin C (1 g), all three nutrients together, or an inactive placebo.

Cancer: Summary

In summary, both biochemical and epidemiologic studies have indicated that antioxidant nutrients and the foods that contain them may have important protective effects in the prevention of human cancer (Table 1). The evidence for a beneficial effect of fruits and vegetables is overwhelming. The evidence for protective effects from individual antioxidant nutrients is less definitive, and therefore other constituents of a diet may also play an important role (Greenwald and McDonald, 1999; Stephens et al., 1996; Van der Vliet, 2000). One major intervention trial, conducted in a non-Western population, has shown a beneficial effect of combined supplementation with ß-carotene, vitamin E and selenium.

In two recently completed trials in Western populations, however, one showed no benefit of supplementation with ß-carotene, vitamin E or vitamin C on prevention of colorectal adenomas, whereas in the other, supplementation with ß-carotene increased the risk of lung cancer in heavy smokers. More solid evidence on the relationship between antioxidant nutrients and cancer may be forthcoming within the next few years from other intervention trials in Western countries (Temple, 2000).

ANTIOXIDANTS AND CARDIOVASCULAR DISEASE

Mechanisms of action

Current theory suggests that oxidation may play a role in cardiovascular disease in two ways, one involving the long-term development of atherosclerosis and the other involving the immediate damage that occurs during a heart attack or stroke. Antioxidants may help counteract both of these processes.

LDL oxidation and atherogenesis

An increasing body of scientific evidence supports the concept that oxidation, possibly mediated by free radicals, may contribute to atherogenesis by transforming low-density lipoprotein (LDL) into an oxidised form. Oxidised LDL has been found in damaged arterial walls

Table 1. Some major intervention trials using antioxidants for cancer prevention.

Country	Cancer site	Study group	Sample size	Treatment	Result	References
China	Oesophagus, stomach and overall mortality	General population of a high risk area	30000	Various combinations of nutrients	(i) Stomach cancer reduction by 21% (ii) Total cancer deaths reduced by 13% (iii) Mortality reduced by 9%	NA
Finland (ATBC cancer study group)	Lung	Male long term smokers	29000	β-carotene and vitamin E (20 and 50 mg/ day respectively)	18% increase in lung cancer incidence	Heinonen et al., 1998
USA	All	Male physicians	22000	β-carotene (50 mg on alternate days)	No effect	Hennekens et al., 1996 a, b
USA (CARET)	Lung	Smokers and asbestos workers	17000	β-carotene (30 mg)and Vitamin A (Retinyl Palmiatae, 25000 IU)	Increase in lung cancer	Goodman et al., 1998
Australa	Skin	General population	1800	β-carotene (550 mg)	No effect	Green et al., 1998
USA	Head and neck	Subjects with previus cancers removed surgically	600	β-carotene	No effect	Mayne et al., 2000
Boston / USA	Breast cancer cells	-	-	Vitamin E	Prevention of tumour development	Schafer and Kerger, 2009
Finland	Prostate cancer	-	-	Vitamin E And catechol	Positive	Heionean et al., 1998
SELECT trial	Prostate cancer	Men	18000	(i) Vitamin E plus selenium (ii) Vitamin E plus selenium placebo (iii) Selenium plus Vitamin E placebo (iv) Double placebo (each tracked for 7-12 years)	(i) Vitamin E offered no prostrate cancer prevention (ii) 17 percent increased risk of cancer among who were assigned to take vitamin E (iii) Non-significant reduction in risk of cancer among men who were assigned to take vitamin E and selenium	Lipmaan et al., 2009; Klein et al., 2011

and has been shown to have several actions that could contribute to the initiation and progression of arterial damage. There is evidence from human studies that associates the extent of LDL oxidation with the extent of atherosclerosis. For example, in a study of Swedish heart attack victims, it was found that increased susceptibility of LDL to oxidation was associated with increased severity of atherosclerosis. Similarly, in a Finnish study, men with accelerated progression of atherosclerosis were found to have higher levels of antibodies to oxidised LDL than men who showed no progression of atherosclorosis. Additional evidence comes from short-term intervention trials in human volunteers, as described below in the Intervention Trials section.

Iscaemia/reperfusion injury

Another way in which oxidants and antioxidants may influence cardiovascular disease is through the process of ischemia/reperfusion injury, which is the immediate cause of tissue damage during a heart attack or stroke. All living tissues of the human body need oxygen to survive. If their supply of blood and oxygen is cut off (a situation called ischemia) they begin to die. Irreversible damage can be prevented only by reperfusion-the restoration of blood flow and reintroduction of oxygen. Ironically, although reperfusion is necessary for recovery, it can damage tissues further, because harmful oxygen free radicals are formed during the reperfusion process. The role of free radicals in heart attacks has been assessed in experimental model systems. Free radicals have been found to accumulate in isolated heart muscle that has been subjected to a temporary interruption of blood flow.

In some experimental models, treatments that inhibit the accumulation of free radicals have been shown to reduce the severity of damage to the heart muscle. In one human study, pretreatment with vitamin C was apparently of benefit to patients who underwent long periods of cardiac arrest during cardiopulmonary bypass surgery. The release of enzymes associated with ischemia was strikingly decreased in those patients who received vitamin C, indicating a reduction in cell damage.

Epidemiologic evidence

An increasing body of epidemiologic evidence links high intakes of antioxidants with reduced risks of cardio-vascular diseases. The evidence is strongest for vitamin E, limited but promising for ß-carotene, and inconsistent for vitamin C.

Vitamin E

The most notable findings on vitamin E come from two large epidemiologic studies conducted in the United States. Key findings of these studies are summarized in Table 2. In an ongoing study of almost 40000 male health professionals, it was found that those in the top fifth of the group in terms of vitamin E intake showed a statistically significant 40% reduction in heart attack risk. In a parallel study of 87000 female nurses, those in the top fifth of vitamin E intake had a 34% reduction in heart attack risk. In both groups, the association was attributable mainly to vitamin E consumed in supplement form. Dietary vitamin E had no significant effect, presumably because vitamin E intakes from food are far lower than those achievable through supplementation. Daily use of single-entity supplements, generally containing at least 100 IU (74 mg ATE) of the vitamin, for 2 or more years was associated with a 37% decrease in heart attack risk among men and a 41% decrease in women. Supplement doses greater than 100–250 IU/day (74–185 mg ATE/day) were not associated with further reductions in risk.

Although these findings are impressive, they do not constitute definitive proof that vitamin E supplementation causes a reduction in heart disease risk. These were not intervention trials; they were observational studies of people who chose for themselves whether or not to use supplements. It is possible that supplement users may have differed from nonusers in other ways that might affect heart disease risk and that these other factors, rather than vitamin intake itself, were responsible for the inverse association between supplement use and coronary risk. However, other aspects of the data argue against this explanation. If supplement use were merely a marker for other aspects of a healthy lifestyle, one would also expect to see effects associated with the use of other types of supplements, including vitamin C. But this was not the case. In both men and women, vitamin C supplementation was not associated with decreased coronary risk. This fact tends to strengthen the case for a specific effect of vitamin E. The idea that vitamin E might protect against heart disease is also supported by other studies.

A cross-cultural study of 16 European population groups (the MONICA study) has shown a strong inverse correlation between blood vitamin E levels and heart disease risk. A study conducted in Scotland showed an inverse correlation between the incidence of angina and blood levels of vitamin E in men. Also, as described below, several trials have shown that high-dose vitamin E supplementation reduces the susceptibility of LDL to oxidation. The EURAMIC study, referred to previously, provides additional evidence about the relationship of vitamin E and other antioxidants to cardiovascular risk. In one arm of this study, vitamin E and ß-carotene concentrations were measured in 683 men who had recently experienced a myocardial infarction and in 727 control men who remained healthy. Coordinated investigations were conducted in nine European centres (Berlin, Germany; Edinburgh, United Kingdom; Granada, Spain; Helsinki, Finland; Malaga, Spain; Moscow, Russia;

Table 2. Some major antioxidant trials for CHD prevention.

Place	Study group	intervention	Risk/ result	References
Harvard school of public health	Middle aged men and women, americans	Vitamin E 100 IU/ D for 10 years	low	Stampfer et al., 1993
-do-	elderly	-do-	low	Losoznsky et al., 1996
United kingdom	Patients with existing heart disease	Vitamin E 400-800 IU/D for 1.5 years	77 percent decrease in heart diseases	Stephens et al., 1996
Finland	Randomly assigned men	Vitamin E (50mg/ D for 12 years on alternate days)	Decrease in heart diseases	Rapola et al., 1996
Harvard school of public health	Nurses health study and Health professionals follow up study	Vitamin E (400 IU or more for 2 years)	20-40 percent reduction in CHD risk	Stampfer et al., 1993 Rimm et al., 1993
GISSI prevention trial	11000 Heart attack survivors	Vitamin E supplements for 3 years	No prevention	Anon, 1999.
HOPE trial	9500 men and women diagnosed with heart diseases or at high risk	Vitamin E supplementation for 3 years	No prevention	Yusuf et al., 2000
Womens health study	40000 women	Vitamin E supplementation (600 IU) for 10 years on alternate days	**(i) Non-significant reduction in major cardiac events** **(ii) 24 percent lower risk of cardiovascular death**	Lee et al., 2005
Physicians health study II	15000 middle aged men, mostly free of heart diseases at start	vitamin E supplements of 400 IU every other day, alone or with vitamin C	No protection against heart attacks, strokes or cardiovascular deaths	Seso et al., 2008

Sarpsborg, Norway; Zeist, The Netherlands; and Zürich, Switzerland) and in Jerusalem, Israel. The researchers found that low adipose tissue vitamin E concentrations were not associated with increased myocardial infarction risk. The lack of effect of vitamin E suggests that protection may be seen only in supplement users. The amounts of vitamin E obtained from foods may be insufficient for protection against myocardial infarction.

ß-Carotene

The EURAMIC study, however, showed that low adipose tissue ß-carotene concentrations were associated with significantly increased risk of myocardial infarction, a risk that was mainly confined to current smokers. The same study of male health professionals that showed an inverse relationship between vitamin E intake and coronary risk also associated high dietary ß-carotene intake with reduced coronary risk in smokers. Current smokers in the top fifth of ß-carotene intake showed a 70% reduction in heart disease risk and former smokers showed a 40% reduction. A preliminary analysis of data from the parallel women's study, reported only in abstract form, also suggests an inverse association between ß-carotene intake and coronary risk. However, these preliminary data show that those in the top fifth of ß-carotene intake (smokers and nonsmokers combined) showed a 22% reduction in heart disease risk.

Vitamin C

As noted above, the U.S. studies of female nurses and male health professionals did not find any association between vitamin C supplementation and coronary risk.

However, several other studies have shown effects of vitamin C. For example, the Basel Prospective Study showed that Swiss men with low blood vitamin C levels had an increased risk of dying from a heart attack during 12 years of follow-up compared with men with normal blood vitamin C levels. Studies comparing different European populations indicate that coronary heart disease mortality is higher in those with blood vitamin C levels that are almost in the deficient range.

An analysis of a 10-year follow-up study of a representative sample of the U.S. population found that men with the highest vitamin C consumption (at least 50 mg/day from diet plus regular use of supplements) had a 42% lower rate of death from cardiovascular diseases and women had a 25% lower rate. Further research is needed to resolve the discrepancy in epidemiologic findings on vitamin C intake and heart disease risk.

Other dietary antioxidants

A small number of studies have suggested that dietary antioxidants other than vitamin E, vitamin C and carotenoids might be protective against heart disease. For example, in a study conducted in The Netherlands, high intakes of flavonoids (found in black tea, onions, and apples) were associated with decreased coronary mortality in a group of elderly men. Garlic also contains antioxidants, and a U.S. study indicated that garlic supplementation can reduce the susceptibility of LDL to oxidation. It has also been suggested that the apparent protective effect of red wine against heart disease, which is believed to contribute to the relatively low cardiovascular death rate in France, may be due not to the alcohol content of the wine but to its antioxidant content. In vitro, antioxidants isolated from red wine have been found to inhibit the oxidation of LDL. The activity of the natural antioxidant ubiquinol is currently under investigation. All of these studies provide intriguing leads for future research.

Intervention trials

As Table 2 indicates three major intervention trials of antioxidants and cardiovascular disease are now in progress, two in the United States and one in the United Kingdom (the MRC/BHF Heart Protection Study, starting date 1994). These studies are likely to provide definitive evidence on the effects of ß-carotene supplementation on cardiovascular risk, and two of the trials should provide valuable information about vitamin E as well. Valuable information has also been obtained from short term trials designed to assess the effect of antioxidant supplementation on the susceptibility of blood lipoproteins to oxidation. In several trials of this type, daily consumption of high- dose vitamin E supplements (800 IU [592 mg ATE] or more), usually for a period of several weeks, has been shown to decrease the susceptibility of LDL from these subjects to in vitro oxidation by copper sulphate. This action appears to be specific to vitamin E; high doses of ß-carotene, another fat-soluble antioxidant, do not have the same effect. Various combinations of antioxidant nutrients have also been shown to be effective in decreasing the oxidation of LDL, but further research is needed to determine whether all of the antioxidants contribute to the effect or whether vitamin E is the only active agent.

Cardiovascular disease: summary

In summary, biochemical studies, epidemiologic investigations and preliminary findings from most intervention trials suggest that antioxidant nutrients may play a role in reducing cardiovascular risk (Losonczy et al., 1996). The evidence is strongest for vitamin E. However, protective effects of vitamin E may be evident only at high doses - much more than can be obtained from a normal diet. Further research is needed to confirm the role of vitamin E and determine the optimal intake. The current evidence on ß-carotene is limited but promising. Clinical trials already in progress should provide definitive answers on the role of this nutrient within the next few years. The evidence on vitamin C and cardiovascular disease is inconsistent, and more study of this nutrient is needed. There is also some limited preliminary evidence suggesting that certain antioxidants other than micronutrient vitamins and minerals in foods may have beneficial effects on cardiovascular health. Results from the Heart Outcomes Prevention Evaluation (HOPE) trial, also showed no benefit of four years worth of vitamin E supplementation among more than 9,500 men and women already diagnosed with heart disease or at high risk for it (Yusuf et al., 2000).

In fact, when the HOPE trial was extended for another four years, researchers found that study volunteers who took vitamin E had a higher risk of heart failure (Lonn et al., 2005). Based on such studies, the American Heart Association has concluded that “the scientific data do not justify the use of antioxidant vitamin supplements [such as vitamin E] for CVD risk reduction” (Kris- etherton et al., 2004).

EYE DISEASES

Age-related diseases of the eye are major health problems around the world. In technologically developed countries, the treatment of cataracts is one of the largest contributors to total health care costs for the elderly. In less developed countries, where treatment is often unavailable, cataracts are a major cause of blindness in older adults. Worldwide, approximately 50 million people are blind from cataracts. In Western countries, the most

common cause of new cases of blindness in the elderly is not cataracts but an age-related disorder of the retina called macular degeneration or Maculopathy. Oxidative processes have been implicated in the causation of both cataract and age related maculopathy. Recent evidence suggests that high dietary intakes of antioxidants may help delay or prevent these disorders.

Cataracts

Cataracts occur when transparent material in the lens of the eye becomes opaque. Much of the material in the lens consists of extremely long-lived proteins, which can become damaged over the decades of a human lifetime. Since there is no direct blood supply to the lens, nutrients enter and waste products are removed by a simple diffusion process which is slow and inefficient. Oxidation, induced primarily by exposure to light, is believed to be a major cause of damage to the proteins of the lens. When these proteins become oxidised, they clump together and precipitate, causing portions of the lens to become cloudy. The eye has defence systems which protect the lens from oxidative damage. Antioxidants and antioxidant enzymes inactivate harmful free radicals and proteases (enzymes which break down proteins) selectively remove damaged proteins from the lens. However, these defense systems cannot always keep pace with oxidative damage. As a result, oxidised proteins may accumulate. As people age, the defence systems grow less effective, and damage to lens proteins may become irreversible.

Several recent epidemiologic studies conducted in Western countries have associated high intakes or blood levels of antioxidant nutrients with reduced rates of cataract. All three of the major dietary antioxidants— vitamin C, vitamin E and carotenoids — have been associated with decreased cataract risk. For example, a Finnish study showed that people with low blood levels of vitamin E or ß-carotene had higher risks of developing cataracts during a 15-year follow-up period. The increase in risk was greatest among individuals who had low blood levels of both of these antioxidant nutrients.

In the ongoing study of female U.S. nurses, it was found that intake of carotene was inversely associated with cataract risk. In the same study, the risk of cataract was decreased by 45% among women who had used vitamin C supplements for 10 years or more. A small Canadian epidemiologic study indicated that nonusers of vitamin C supplements were 3.3 times more likely than users to develop cataracts. Similarly, nonusers of vitamin E supplements were 2.3 times more likely than users to develop cataracts. A multicentre U.S. study found that use of multivitamins was associated with a decreased risk of each of four major types of cataract; high intakes of fruits and vegetables also appeared to be protective. In another U.S. study, low blood vitamin C levels were associated with an 11-fold increased risk of one type of cataract, and low blood carotenoid levels were associated with a 7-fold increased risk of another type of cataract. Low intakes of fruits and vegetables were also associated with increased cataract risk. In contrast, 2 studies conducted in the Orient had negative results. An epidemiologic study conducted in fishing communities of Hong Kong found no significant associations between blood levels of antioxidant nutrients and cataract. Also, in the recent nutrition intervention trial in Linxian, China, supplementation with ß-carotene/vitamin E/selenium or with vitamin C/molybdenum did not lead to reductions in cataract risk. However, it should be noted particularly in the Hong Kong study, the subjects were probably exposed to high levels of ultraviolet radiation — a major causative factor in cataract.

Interestingly, a riboflavin/niacin supplement did show a significant protective effect, which may be indirectly related to antioxidant defence mechanisms. Riboflavin is a cofactor for the activity of several essential antioxidant enzymes, and intakes of this vitamin in Linxian are marginal at best. The riboflavin/niacin supplement may have exerted its effect by correcting a subclinical riboflavin deficiency, thus enhancing the activity of antioxidant enzymes. However, a similar benefit of riboflavin supplementation would not be expected in Western populations, where intakes of this vitamin are generally ample. The difference between the Western and Asian findings may reflect differences in overall nutritional status and cataract risk factors in the two types of populations

Age-related macular degeneration

Some scientific evidence suggests that excessive exposure to light and the resulting production of oxidants contribute to the causation of age-related maculopathy. In particular, exposure to blue light has been implicated in this disorder. If blue light is involved, carotenoids might be protective, since they can absorb blue light. An analysis of data from a national U.S. survey showed an inverse correlation between age-related maculopathy and the consumption of "vitamin A-rich" fruits and vegetables. The "vitamin A" found in these foods actually consists of carotenoid precursors of the vitamin rather than preformed vitamin A (retinol). A large multicentre U.S. case-control study that focused on the most severe form of age-related maculopathy found markedly reduced risks in individuals with high blood carotenoid levels. Significant associations were detected for the sum of all carotenoids and for four of five individual carotenoids (ß-carotene, α-carotene, cryptoxanthin and lutein/zeaxanthin, but not lycopene). Vitamin C and vitamin E did not show significant effects. In contrast, a much smaller British study that included all types of age-related macular degeneration found no association between serum carotenoid or vitamin E levels

Table 3. Some major antioxidant trials for eye disease prevention.

Place	Experimental population	intervention	Result	References
USA	US women	Vit C supplement for 10 years or more	45% lower catarct incidence	Hankinson et al., 1992
USA	US physicians	multivitamins	25% lower catarct incidence	Seddon et al., 1994
USA	Elderly men	Multivitamin supplements or vitamin E	Lower risk of opquenes of lens	Leske and Chylack, 1991
Canada	Non-users of vitamin E	-	3.3 times more likely to develop cataract	Robertson et al., 2002
Canada	Non users of vitamin E	-	2.3 times more likely to develop catarct	-do-
Finland	Smokers	Vit E/ β carotene for 6 years	No effect	Tiekari et al., 1997

and the risk of macular degeneration. The difference between these findings and those of the U.S. study may reflect differences in sample size or differences in the pathogenesis of the different types of maculopathy.

In summary, there is evidence suggesting that oxidative processes may play a role in causing age-related disorders of both the lens and the retina of the eye. All of the published epidemiologic studies of antioxidants and cataract in Western populations have shown significant associations for at least one antioxidant nutrient. The overall body of evidence suggests that all three major dietary antioxidants — vitamin C, vitamin E and carotenoids — may be beneficial in reducing cataract risk in Western populations. Less is known about age-related macular degeneration, but some epidemiologic evidence suggests an inverse association with carotenoids. Further research is needed to determine conclusively whether improved nutrition may reduce the risk of these very prevalent eye disorders in older adults (Table 3).

OTHER DISEASES AND PATHOLOGICAL PROCESSES

Although most research on oxidants, antioxidants and disease has focused on cancer, cardiovascular disease and degenerative disorders of the eye, oxidative processes are also implicated in a wide variety of other clinical conditions and pathological processes, as shown in Table 4. A few examples of particular interest are described below.

Neurological disorders

Biochemical studies suggest that oxidation may play a role in the causation of several disorders of the brain and nervous system. Therefore, it has been hypothesized that antioxidants might be helpful in ameliorating the symptoms or in slowing the progression of some neurological disorders. Some studies have shown beneficial effects of vitamin E supplementation in decreasing the severity of Tardive dyskinesia; others have not found an effect. Tardive dyskinesia is a disorder involving involuntary movement. It occurs as a side effect of long-term treatment with certain antipsychotic drugs. An open pilot trial indicated that supplementation with vitamins C and E might be of benefit in slowing the progression of Parkinson's disease. However, a larger placebo-controlled trial found no benefit from vitamin E therapies.

Sperm damage, birth defects and childhood cancer

The children of fathers who smoke cigarettes have increased rates of congenital malformations and childhood cancer. These problems may be related, at least in part, to increased oxidative damage to sperm cells caused by oxidants in cigarette smoke. Cigarette smoking is associated with reduced sperm count and poor sperm quality; it is also associated with decreased blood vitamin C levels. Vitamin C supplementation has been shown to improve sperm quality in heavy smokers. Ample intakes of vitamin C have also been shown to reduce oxidative damage to sperm DNA. Further studies are needed to determine whether improved antioxidant status, particularly with respect to vitamin C, is of benefit in reducing infertility and germ-line mutations in men who smoke cigarettes or who are exposed to oxidative stress from other causes.

Exercise-induced oxidative stress

Because exercise leads to increased oxygen

Table 4. Some major antioxidant trials for neurological disease prevention.

Place	Experimental population	Intervention	Results	References
USA	Patients with moderately severe Alzheimer's disease	2000 IU/ D Vit E or placebo for 2 – 4 years	Treated patients were better (non-significantly)	Sano et al., 1997
USA	Patients with moderately severe parkinsons' disease	Vit E supplementation for 5 years	No effect	Agid et al., 1993

consumption, it causes an increase in the production of oxygen-initiated free radicals. Some studies have reported that antioxidant supplementation reduces evidence of exercise-induced muscle damage. Antioxidants may also play a role in reducing muscle soreness after overexertion. However, it is unclear whether antioxidants can directly enhance physical performance.

Inflammatory disorders

Free radicals and oxidative stress may play a role in inflammatory diseases. Rheumatoid arthritis is one example. The products of free radical reactions have been detected in the blood and joints of patients with this disease. Other lines of evidence also suggest the involvement of oxidative stress in rheumatoid arthritis and in other inflammatory diseases such as glomerulonephritis.

Decreases in immune function

Several aspects of immune function show a marked decline with increasing age. Preliminary studies in elderly people have indicated that this decline can be partly offset by dietary antioxidant supplementation. The age-associated decrease in cell-mediated immunity may be due to a decreased level of small-molecular-weight antioxidants and decreased activity of antioxidant enzymes.

OXIDANTS, ANTIOXIDANTS, AND DISEASE: IMPLICATIONS OF THE SCIENTIFIC EVIDENCE

Although much remains to be learned, there is now convincing evidence for the involvement of free radicals in a number of diseases which constitute major public health problems throughout the world. Free radicals and oxidative processes are believed to play important roles in the pathogenesis of many age-related disorders. The evidence implicating them in the causation of cancer, atherosclerotic cardiovascular disease and cataracts is especially strong. The human body has a complex system of natural enzymatic and nonenzymatic antioxidant defences which counteract the harmful effects of free radicals and other oxidants. Protection against free radicals can be enhanced by ample intakes of dietary antioxidants, of which the best studied are Vitamin E, C and carotenoids. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention. Efforts should be made to ensure optimum intakes of foods containing these important molecules.There is a need for improvement in the quality of the diet, especially with respect to increased consumption of fruits and vegetables.

However, other strategies, including optimization of food processing, selective fortification of foods and the use of safe nutritional supplements, may also need to be considered. All three of the major antioxidant nutrients-vitamin E, vitamin C and ß-carotene-are safe even at relatively high levels of intake (Berger et al., 2012). There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases.

In addition, there is a potential for substantial savings in the cost of health care delivery. More research is needed to clarify and extend scientific understanding of the health effects of antioxidants. Basic research should continue, and additional large-scale randomised trials and clinical studies should be undertaken. Funding for research in this field is urgently needed. At the same time, efforts should also be made to communicate to the general public on existing information about the importance of protective nutrients in fruits and vegetables. Government agencies, health professionals and the news media should work together to promote the dissemination of scientifically sound information about this aspect of nutrition to all segments of the population.

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