

# CURRENT RESEARCH

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## The Potential Value of Natural Antioxidative Treatment in Glaucoma

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**Abstract.** Glaucomatous optic neuropathy implies loss of retinal ganglion cells, including their axons, and a major tissue remodeling, especially in the optic nerve head. Although increased intraocular pressure is a major risk factor for glaucomatous optic neuropathy, there is little doubt that other factors such as ocular blood flow play a role as well. Mechanisms leading to glaucomatous optic neuropathy are not yet clearly understood. There is, however, increasing evidence that both an activation of glial cells and an oxidative stress in the axons play an important role. Glial cells may be activated by mechanical stress via activation of the epidermal growth-factor-receptor, or by ischemic stress via an increase in endothelin. Several factors can systemically or locally increase oxidative stress. In glaucoma, an unstable ocular blood flow leading to repeated mild reperfusion seems to be most relevant in inducing oxidative stress. The simultaneous production of nitric oxide in the astrocytes and of superoxide in the mitochondria of the axons leads to the production of the damaging peroxynitrite. Therapeutically, we need to reduce intraocular pressure, stabilize ocular blood flow, and reduce oxidative stress. Various natural compounds possess potential antioxidative value. Reduction of oxidative stress at the level of mitochondria can be achieved by ginkgo biloba. Polyphenolic compounds, such as tea, red wine, dark chocolate, or coffee have antioxidative properties. Coffee contains 3-methyl-1,2-cyclopentanedione (MCP), capable of scavenging peroxynitrite. Red wine-polyphenols (e.g., resveratrol), exert vasoprotective effects by inhibiting the synthesis of endothelin-1. Dark chocolate decreases blood pressure and improves endothelium-dependant vasorelaxation. Anthocyanosides (bilberries) owe their antioxidant effects to their particular chemical structure. Other antioxidants include ubiquinone and melatonin. (*Surv Ophthalmol* 53:479–505, 2008. © 2008 Elsevier Inc. All rights reserved.)

**Key words.** antioxidants • glaucoma • neuroprotection • pro-oxidants • trabecular meshwork

Ophthalmologists are at times confronted with questions from patients regarding alternative natural nutritional sources for eye health. This may partly be due to the fact that patients are often dissatisfied with the conventional form of treatment provided because it is believed to be ineffective,<sup>12</sup> has produced adverse effects,<sup>14,144</sup> is too technolog-

ically oriented, or because it is less compatible with the patient's values regarding nature and meaning of health and diseases.<sup>301</sup> The impact of nutrition on the manifestation and progression of ocular diseases has thus become an important but controversial topic. Interest has been directed to the potential value of antioxidant treatment in ocular

diseases where oxidative stress has been implied to play a role.<sup>207,239</sup>

The eye is a unique organ because of its constant exposure to light, atmospheric oxygen, environmental chemicals, and physical abrasion. Metabolism of oxygen by cells generates potentially deleterious reactive oxygen species (ROS) (Fig. 1).<sup>256</sup> Under optimal conditions the rate and magnitude of oxidant formation is balanced by the rate of oxidant elimination through the action of antioxidants. But even under normal conditions excess oxidants may cause macromolecular damage. An imbalance between pro-oxidants and antioxidants, in favor of the former, results in oxidative stress.<sup>265</sup> This insult, in turn, may lead to damage of a variety of macromolecules, such as proteins, lipids, sugar residues, or DNA, and thereby leads, in extreme cases, to growth arrest, growth modulation, or cell death, such as death of retinal ganglion cells in glaucoma.<sup>58</sup> As oxidative stress has been proposed to play a role in the pathogenesis of glaucomatous optic neuropathy,<sup>7,97,140,141,179,187,215</sup> the possibility that antioxidant balance may be manipulated through diet has therefore created much interest.

Theoretically, the intake of nutritional antioxidants has a beneficial impact on glaucoma. If increasing total dietary antioxidant intake is shown to be beneficial, this would be a promising means of therapeutic prevention of further damage in glaucoma. However, this hypothesis has not been evaluated thoroughly.<sup>151,152,299</sup> As such, the potential beneficial value of nutritional factors in glaucoma is insufficiently understood.

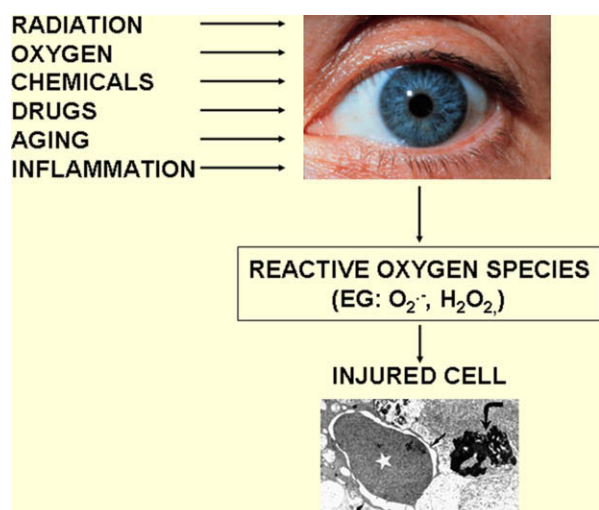


Fig. 1. The eye is an organ that is predisposed to great levels of oxidative stress. The eye is constantly exposed to factors such as radiation, chemicals, oxygen, and drugs, which induce the formation of reactive oxygen species (ROS) that can ultimately damage cells.

This review aims to assess the role of oxidative stress in the pathophysiology of glaucoma and to summarize some of the most common nutrients with antioxidative properties, keeping in mind the possible benefits of antioxidant nutrition in the oxidative-related pathophysiology of glaucoma.

### Basics of Redox Chemistry and Terminology

The term *oxidation* refers to the loss of electrons (Table 1); pro-oxidants and their compounds are those which can oxidize other molecules. Pro-oxidants can be in the form of free radicals or non-radical species. Free radicals are molecules that contain one or more unpaired electrons. The presence of an unpaired electron sometimes makes free radicals highly reactive (the need to “pair up” the unpaired electron), although the chemical reactivity of radicals varies over a wide spectrum. Pro-oxidants are molecules with a strong oxidizing potential (e.g., hydrogen peroxide H<sub>2</sub>O<sub>2</sub>, ozone O<sub>3</sub>) or species that favor the formation of strong oxidants (e.g., transition metals) (Table 2). Radical and non-radical molecules can in turn be derived from oxygen (ROS); or from nitrogen (reactive nitrogen species, RNS).

### The Oxygen Molecule

#### HISTORY OF THE OXYGEN MOLECULE

Oxygen appeared in significant amounts in the earth’s atmosphere more than a billion years ago; geological evidence suggests that this was due to the evolution of photosynthesis by blue-green algae (cyanobacteria).<sup>93,155</sup> Cyanobacteria are photosynthetic and aquatic. As they split water to obtain the hydrogen needed to drive metabolic reductions these bacteria released tons of oxygen in the atmosphere.

TABLE 1

#### Basics of Redox Chemistry and Terminology

Oxidation	Gain in oxygen Loss of electrons Loss of hydrogen
Reduction	Loss of oxygen Gain of hydrogen Gain of electrons
Oxidizing agent	Oxidizes another chemical by taking electrons from it, or by taking hydrogen, or by adding oxygen
Reducing agent	Reduces another chemical by supplying electrons to it, by supplying hydrogen, or by removing oxygen

TABLE 2  
*Pro-oxidants*

Free radical	Species capable of independent existence that contain one or more unpaired electrons (e.g., R <sub>3</sub> N, R-O)
Non radicals	Species that have strong oxidizing potential (e.g., H <sub>2</sub> O <sub>2</sub> , O <sub>3</sub> )
	Species that favor the formation of strong oxidants (transition metals, e.g., Me <sup>2+</sup> )

When living organisms first appeared on earth, they did so under an atmosphere containing very little oxygen (i.e., they were anaerobes).<sup>70</sup> Eukaryotic organisms began the evolutionary process of using and adapting to the environmental changes brought about by appearance of oxygen. Eukaryotic organisms were naturally selected over others. These organisms developed antioxidant defences and evolved the electron transport chain (ETC), where oxygen was used as the terminal electron acceptor, enabling the oxidation of food more efficiently.<sup>63,114</sup> The evolution of the ETC was brought about by endosymbiosis of bacteria (mitochondria are descendants of bacteria) in eukaryotic host cell (Fig. 2).

**THE OXYGEN PARADOX**

The paradox of aerobic life,<sup>51</sup> or the *oxygen paradox*, is that higher eukaryotic aerobic organisms cannot exist without oxygen, yet oxygen is dangerous to the very lifeforms for which it has become an essential component of energy production. This is related to the fact that each oxygen atom in the oxygen molecule has one unpaired electron in its outer valence shell, and molecular oxygen has two unpaired electrons. Thus, atomic oxygen is a free radical and molecular oxygen is a (free) bi-radical.<sup>85</sup> The tetravalent reduction of molecular oxygen by the mitochondrial electron-transport chain pro-

duces water; however, the system does not always work perfectly, and as a result of electron leakage, the univalent reduction of oxygen molecule generates reactive intermediates.<sup>161,304</sup> The leakage generates superoxide anions (O<sub>2</sub><sup>-</sup>) (Fig. 3).<sup>276</sup> At physiological oxygen levels it has been suggested that about 1–3% of the oxygen reduced in mitochondria may form the superoxide anion.<sup>28,118</sup>

**OXYGEN MOLECULE IN GROUND STATE**

The diatomic oxygen molecule in ground state is a free radical. A molecule in ground state is not energetically excited. Ironically, although the oxygen molecule is a di-radical, and most free radicals are generally highly reactive, this molecule is minimally reactive. The oxygen molecule has two unpaired electrons, each of which are located in different π\* antibonding orbitals (Fig. 4). These two electrons rotate about their own axis in the same direction and thereby have the same or parallel spins. This is the most stable state or ground state of oxygen. If oxygen accepts two electrons (gets reduced) from another atom or molecule, both of these electrons must be of antiparallel spin, to oxygen's electrons, so as to fit in to the vacant spaces in the π\* orbitals. This makes it difficult for the oxygen molecule to accept a pair of electrons from another atomic or molecular orbital, because a pair of electrons in other atomic or molecular orbitals would have opposite spins. As a result of this spin restriction on electron transfer, oxygen is forced to accept its electrons one at a time, explaining why this molecule reacts sluggishly with many non-radicals.<sup>83</sup>

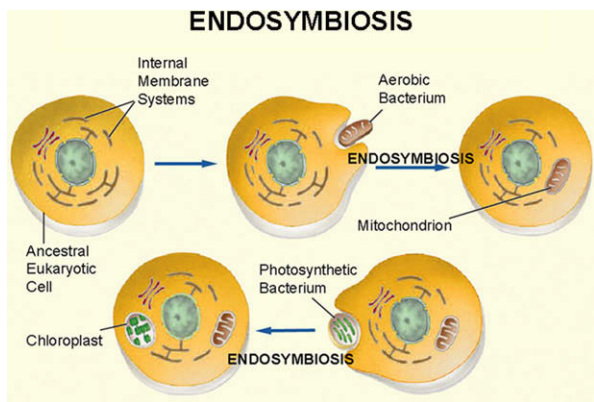


Fig. 2. The mitochondria of eukaryotes evolved from ancient endosymbiosis of aerobic bacteria by the ancestral eukaryotic cell.

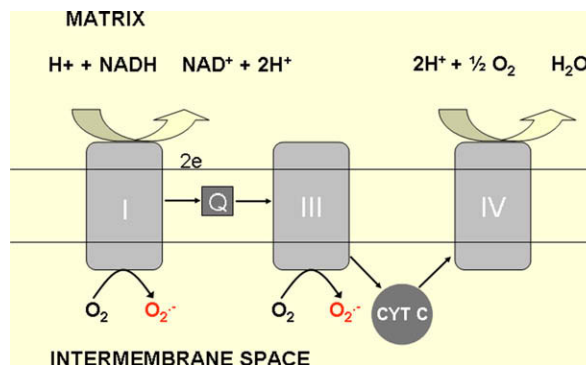


Fig. 3. The electron transport4 chain allows efficient energy production by transferring electrons from donors such as NADH to oxygen (the terminal electron acceptor). However, electron transport does not always function perfectly: various redox centres may leak an electron to oxygen, while passing the great bulk of electrons onto the next component in the chain. This electron leakage may generate the superoxide anion (O<sub>2</sub><sup>-</sup>).

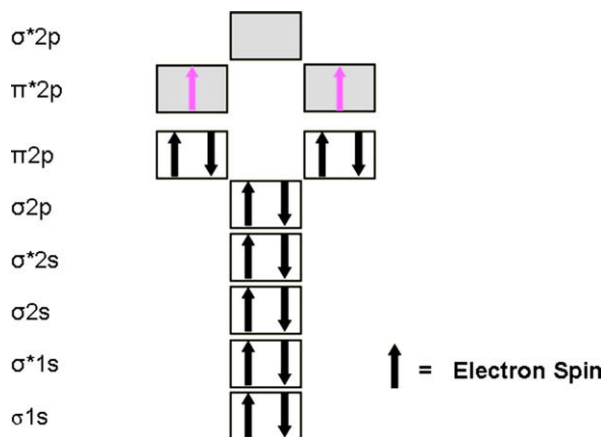


Fig. 4. Bonding diagram of the diatomic oxygen molecule. Atomic oxygen (atomic number 8) has a total of eight electrons. The oxygen molecule (O<sub>2</sub>) has 16 electrons. In the ground state (the most stable state of oxygen), the last two electrons of the oxygen molecule are located in a different π\* antibonding orbital. These two unpaired electrons have the same quantum spin number (they have parallel spins) and qualify ground state oxygen to be a di radical.

**ACTIVATION OF OXYGEN**

Activation of oxygen may occur by two different mechanisms; either through the absorption of sufficient energy to reverse the spin on one of the unpaired electrons, or through monovalent reduction (Fig. 5). If ground state oxygen absorbs sufficient energy to reverse the spin of one of its unpaired electrons, the two unpaired electrons now have opposite spins. This activated form of oxygen is called singlet oxygen (<sup>1</sup>O<sub>2</sub>). Singlet oxygen is much more reactive than ground state oxygen and reacts particularly destructively with molecules with double bonds.<sup>27</sup> The second mechanism of activation is by the step-wise monovalent reduction of oxygen giving

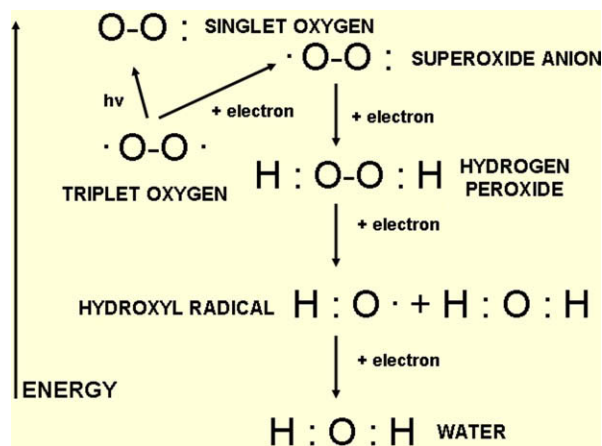


Fig. 5. Activation of oxygen occurs either through absorption of sufficient energy or through monovalent reduction.

rise to superoxide anion radical (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>),<sup>143</sup> and finally to water (H<sub>2</sub>O).

**Major Molecules Involved in Oxidative Stress**

**FREE RADICAL PROOXIDANTS**

**Reactive Oxygen Species (ROS)**

*Singlet Oxygen*

Singlet oxygen is generated mostly by photodynamic reactions. The prerequisite for photodynamic reactions is photosensitization. If certain molecules are illuminated with light of adequate wavelength, they are capable of absorbing this light, which shifts these molecules into an excited state. This excitation energy can then be transferred onto an adjacent oxygen molecule, converting it to the singlet state while the photosensitizer molecule returns to its ground state. Singlet oxygen can exist in the more (157 KJ above ground state) and less (93.6 KJ above ground state) reactive forms (Fig. 6).

**Superoxide Anion**

Superoxide anion (O<sub>2</sub><sup>-</sup>) is generated continuously by cellular processes including mitochondrial electron transport systems. Cells of the immune system (neutrophils, macrophages) also produce the superoxide anion because they contain a membrane-bound enzyme complex, the NADPH oxidase,<sup>62</sup> that reduces oxygen to superoxide anion. Once the superoxide anion is formed, it can be converted to hydrogen peroxide by the enzyme superoxide dismutase. The enzyme myeloperoxidase can con-

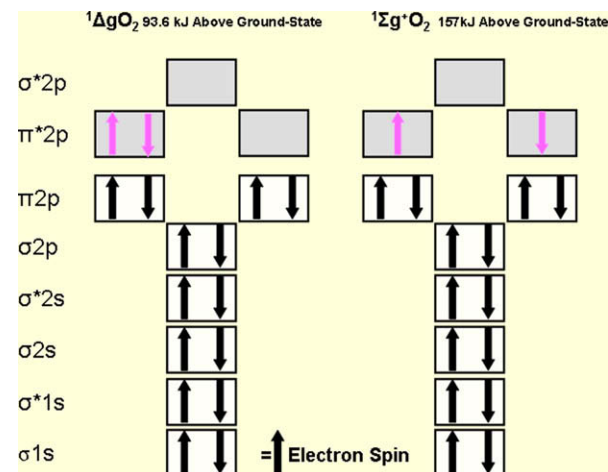


Fig. 6. More reactive forms of oxygen or singlet oxygens are formed when ground state oxygen absorbs sufficient energy. The <sup>1</sup>ΔgO<sub>2</sub> state of the singlet oxygen has an energy 93.6 kJ above ground state. The <sup>1</sup>Σg+O<sub>2</sub> state is even more reactive, 157 kJ above the ground state.

vert hydrogen peroxide to hypochlorous acid, which can further react with the superoxide anion to form the hydroxyl radical.<sup>157,317</sup> These final products are essential for effective bacterial killing (Fig. 7A).

The rapid disappearance of the superoxide anion in aqueous solution is due to dismutation reaction (Fig. 7B).<sup>139</sup> Therefore, any biological system that generates superoxide anions will also produce hydrogen peroxide by superoxide dismutation.

### Hydrogen Peroxide

The oxidizing potential of hydrogen peroxide mixed with ferrous salts was first described by

Fenton in the late nineteenth century (Fig. 7B).<sup>240,287</sup> Hydrogen peroxide is only a weak oxidizing and reducing agent. For example, no oxidation occurs when DNA, lipids, or most proteins are incubated with hydrogen peroxide, even at millimolar levels. Most of the cellular damage done is mediated by the hydroxyl radical (OH·), which is formed after hydrogen peroxide has crossed cell membranes and has reacted with copper or iron ions.<sup>271</sup> Most ROS are so reactive and short-lived, that they are difficult to measure directly (Fig. 8).

### REACTIVE NITROGEN SPECIES (RNS)

#### Nitric Oxide and Peroxynitrite

Nitric oxide (NO) is produced in biological tissue by nitric oxide synthase (NOS) which acts as a catalyst to convert L-arginine and oxygen to nitric oxide and L-citrulline.<sup>95</sup> Nitric oxide is an uncharged lipophilic, free radical molecule that contains a single unpaired electron in the outer valence of its oxygen constituent.<sup>56</sup> Nitric oxide reacts rapidly only with a selected range of molecules that also have an unpaired electron in their outer orbital shell.<sup>138</sup> Nitric oxide strongly reacts with superoxide (O<sub>2</sub><sup>-</sup>) to form peroxynitrite (ONOO<sup>-</sup>) (Fig. 9).<sup>213</sup> Nitric oxide has both pro- and antioxidant properties. A nitric oxide radical, for example, can both stimulate<sup>269</sup> and inhibit lipid oxidation.<sup>136</sup>

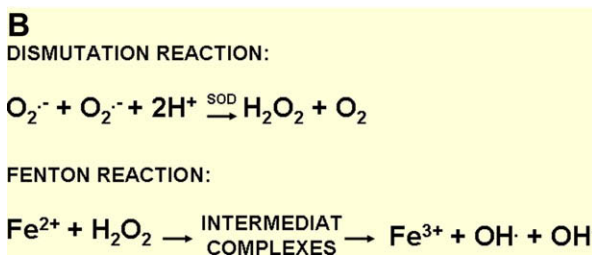
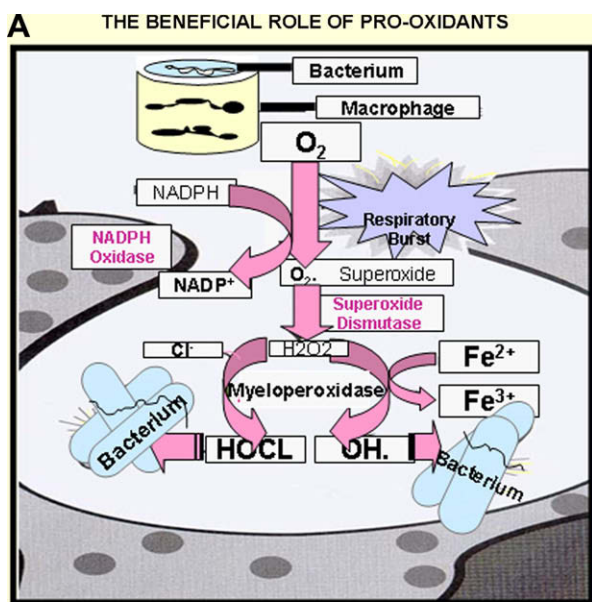


Fig. 7. A: Pro-oxidants have a beneficial role in killing bacteria as shown by their production during respiratory burst. B: Dismutation reaction is any chemical reaction of the type  $A + A \rightarrow A' + A''$ , where A, A' and A'' are different chemical species. The enzyme superoxide dismutase (SOD) catalyzes the dismutation of two molecules of superoxide anion into hydrogenperoxide and oxygen. Fenton discovered in 1984 that several metals have oxygen transfer properties which improve the use of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Fenton reaction is a reaction between iron II and hydrogen peroxide in aqueous solutions. Hydrogen peroxide oxidizes iron II (Fe<sup>3+</sup>) and in the process generates the highly reactive hydroxyl radical (OH·). Nowadays, Fentons reaction is used to treat a large variety of water pollution such as phenols, formaldehyde, or pesticides.

### Major Endogenous Sources of Pro-oxidants

#### MITOCHONDRIA

#### Evolution and Dual Funtion of Mitochondria

Mitochondria are believed to behave like aerobic bacterial cells.<sup>59,104</sup> The theory why a bacterium became a cellular organelle is believed to be linked to the increase in ambient oxygen tension in earth's atmosphere approximately two billion years ago.<sup>231</sup>

ROS	Symbol	Half-Life Time
Hydroxyl	·OH	one nanosecond
Singlet Oxygen	<sup>1</sup> O <sub>2</sub>	one microsecond
Superoxide	·O <sub>2</sub> <sup>-</sup>	one microsecond
Alkoxy	·OL	one microsecond
Peroxy	LOO·	ten milliseconds
Nitric Oxide	·NO	few seconds

Fig. 8. Most reactive oxygen species (ROS) have very short half-lives at 37°C that vary from a few nanoseconds to a few seconds, which makes it difficult to measure them.

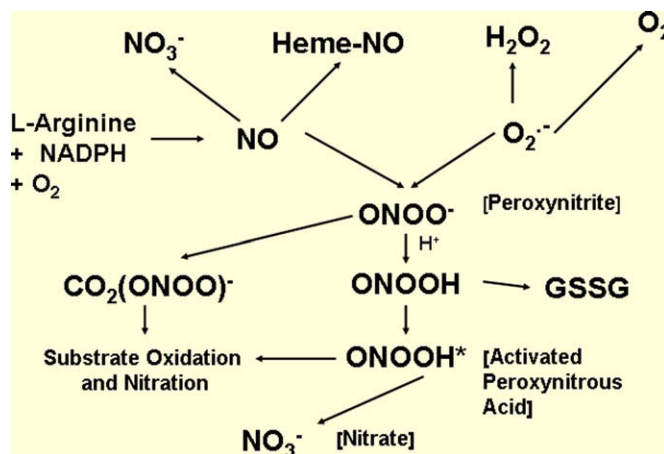


Fig. 9. The chemical reaction between nitric oxide (NO) and the superoxide anion from the highly damaging peroxynitrite. Addition of a proton to the peroxynitrite ( $\text{ONOO}^-$ ) leads to the formation of peroxynitrous acid ( $\text{ONOOH}$ ). Activated peroxynitrous acid ( $\text{ONOOH}^*$ ), or the excited state of peroxynitrous acid, can either lead to substrate oxidation and nitration or simply rearrange to nitrate ( $\text{NO}_3^-$ ) in the absence of adequate substrate.

This environmental trauma is thought to have pushed the symbiosis that lead mitochondria organelles in eukaryotes.<sup>171</sup>

Mitochondria have a dual, even paradoxical effect on the cellular health,<sup>256</sup> in that they not only provide cells with an energy source of ATP, but favor the formation of free radicals, and thereby induce apoptosis (Fig. 10A).<sup>24</sup> Probably the most important source of superoxide anion is a result of electron leakage<sup>24,292</sup> from the electron transport chain in aerobic cells.

### Endoplasmic reticulum

The endoplasmic reticulum contains a group of enzymes that are collectively known as cytochrome P450.<sup>246</sup> Cytochromes P450 are involved in the oxidation of a wide range of substrates at the expense of oxygen.<sup>67</sup>

### Nucleus

The membrane surrounding the nucleus contains an electron transport chain, of unknown function, which receives electrons from NADH or NADPH. This electron transport does not always function perfectly and may leak electrons to the oxygen molecule to form the superoxide anion.<sup>284</sup>

### Cytoplasm

The enzymes xanthine oxidase and xanthine dehydrogenase, found in the cytoplasm, are involved in the metabolism of purines and pyrimidines to uric acid.<sup>52</sup> Xanthine oxidase and xanthine dehydrogenase are complex metalloflavoproteins that react with molecular oxygen and  $\text{NAD}^+$  (Fig. 10B).<sup>128</sup> The metabolism of the purines,

xanthine and hypoxanthine, by the enzyme xanthine dehydrogenase normally does not produce superoxide anions in vivo.<sup>116</sup> However, the conversion of xanthine dehydrogenase to xanthine oxidase,<sup>65</sup> which occurs during ischemia or reperfusion damage,<sup>218,330</sup> can lead to the production of the superoxide anion in vivo.

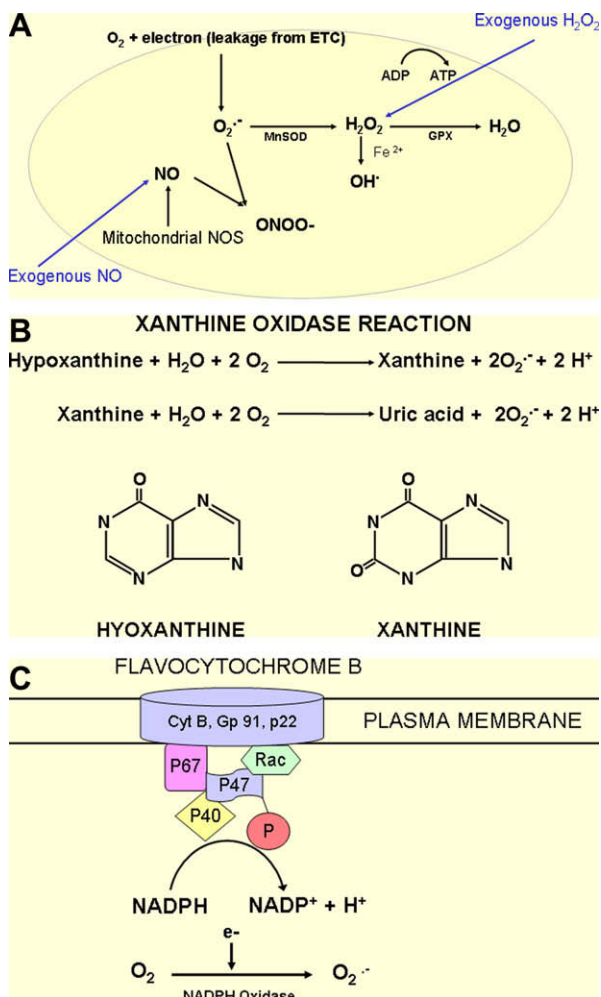
### Plasma membrane

The rapid generation of ROS such as the superoxide anion by the enzyme NADPH oxidase located in the plasma membrane is considered to be a defense response. ROS can serve as direct protective agents by their toxicity against invading pathogens. The enzyme NADPH oxidase catalyses the production of the superoxide anion from the oxygen molecule using NADPH as a reducing agent. In a resting cell, the components of the NADPH oxidase are present in two different compartments of the cell, namely the cell membrane (a flavocytochrome b) and the cytoplasm (various water soluble proteins such as Rac1).<sup>202,300</sup> These proteins can assemble to the membrane-bound flavocytochrome b (consisting of cytochrome b and proteins gp91 and p22), which in turn becomes activated and can generate the superoxide anion from oxygen through the NADPH oxidase activity (Fig. 10C).<sup>115,300</sup>

## The Antioxidant Defense System

### DEFINITION

An antioxidant is by definition any substance that, when present at low concentration compared with those of an oxidizable substrate, significantly delays



**Fig. 10.** A: Mitochondrial sources of pro-oxidants. The main source of superoxide anion ( $\text{O}_2^{\cdot-}$ ) in vivo is the electron transport chain (ETC). Certain components of the mitochondrial ETC do leak a few electrons directly onto oxygen ( $\text{O}_2$ ), while passing the great bulk of electrons onto the next component in the chain. This leakage generates the superoxide anion ( $\text{O}_2^{\cdot-}$ ). B: The enzyme xanthine oxidase catalyses the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. The latter reaction leads to the formation of the superoxide anion ( $\text{O}_2^{\cdot-}$ ). C: The enzyme NADPH oxidase catalyses the production of the superoxide anion from the oxygen molecule using NADPH as a reducing agent. In a resting cell, the components of the NADPH oxidase are present in two different compartments of the cell, namely the cell membrane and the cytoplasm. The membrane-bound component, a flavocytochrome b, is the catalytic core of the NADPH oxidase. The cytosolic components are various water soluble proteins, namely p67 phox, p47 phox, p40 phox and Rac 1. These proteins can assemble to the membrane-bound flavocytochrome b (cytochrome b and proteins Gp91 and p22), which in turn becomes activated and can generate the superoxide anion from oxygen through the NADPH oxidase activity.

or prevents oxidation of that substrate).<sup>117</sup> The antioxidant defense system comprises a variety of molecules: enzymes, such as superoxide dismutase,<sup>106</sup> catalase,<sup>31,42,90</sup> or glutathione peroxidase,<sup>125</sup> that are capable of catalytically removing free radicals and other reactive species (Fig. 11, Table 3); proteins, such as transferrins or haptoglobins,<sup>22</sup> that minimize the availability of pro-oxidants such as iron or copper ions; heat shock proteins that protect biomolecules against damage;<sup>72</sup> and low-molecular-mass molecules, such as alpha-tocopherol, ascorbic acid, or glutathione,<sup>9,182,195,228</sup> capable of scavenging ROS and RNS. The composition of antioxidant defences differs from tissue to tissue and from cell-type to cell-type.<sup>297</sup>

### EXAMPLES OF ENZYMATIC ANTIOXIDANTS AND THEIR REACTIONS

#### Superoxide Dismutase (SOD)

Superoxide dismutase is an enzyme known to catalyze the dismutation of superoxide to hydrogen peroxide and oxygen.<sup>244</sup> It is now generally accepted that the biological role of SOD is to scavenge the superoxide anion.<sup>130</sup> In humans three forms of superoxide dismutase are present which are classified on the basis of the metal cofactor.<sup>166</sup>

#### Catalase (CAT)

Catalase is a heme-containing enzyme that catalyzes the dismutation of hydrogen peroxide to water and oxygen.<sup>227</sup> It is a water-soluble enzyme that is usually found in peroxisomes.<sup>263</sup> Catalase provides a protective role that is similar to that of glutathione peroxidase because both are important means of removing hydrogen peroxide.

#### Glutathione Peroxidase (GPX)

Glutathione peroxidase (GPX) is a cytoplasmic and mitochondrial enzyme that reduces hydrogen peroxide to water by oxidizing glutathione (GSH).<sup>295</sup> Re-reduction of the oxidized form of glutathione (GSSG) is then catalyzed by the enzyme glutathione reductase.

### EXAMPLES OF NON-ENZYMATIC ANTIOXIDANTS AND THEIR REACTIONS

#### Glutathione

Glutathione (g-glutamylcysteinylglycine, [GSH]) is a sulfhydryl (-SH) antioxidant, composed of the three amino acids L-cysteine, L-glutamic acid, and glycine (Fig. 12A).<sup>135,319</sup> The strong antioxidative property of glutathione is due to sulphur atom in the sulfhydryl groups, which enables glutathione to donate electrons readily.<sup>198</sup> When thiols such as

TABLE 3  
*Various potential antioxidative agents*

Various Potential Antioxidative Agents	Substance	Mechanism of Protection	Examples of studies supporting antioxidant effects	In vitro	In vivo	Clinical
Tea	Polyphenolic flavonoids	1. Free radical scavenging properties 2. Iron chelating properties	Wei H et al (1999) <sup>314</sup> Morel I et al (1993) <sup>206</sup>	+		
Coffee	Polyphenolic flavonoids MCP	1. Free radical scavenging properties 2. Selective scavenger of peroxynitrite	Daglia M et al (2004) <sup>48</sup> Kim AR et al (2006) <sup>160</sup>	+		
Wine	Polyphenolic flavonoids Anthocyanins	1. Free radical scavenging properties 2. Enhances the production of vasodilating factors 3. Inhibits the production of VEGF	Rice-Evans CA et al (1996) <sup>235</sup> Leikert JF (2002) <sup>175</sup> Fuhrman B (2001) <sup>87</sup>	+		+
Ginkgo biloba	Polyphenolic flavonoids Terpenoids	1. Free radical scavenging properties at mitochondrial level 2. Blood flow enhancer	Garvin S (2006) <sup>94</sup> Eckert A et al (2005) <sup>61</sup> Hirooka K et al (2004) <sup>129</sup> Chung HS et al (1999) <sup>43</sup> Quaranta et al (2003) <sup>229</sup> Bagchi et al (2006) <sup>17</sup>	+		+
Bilberry	Anthocyanin	Free radical scavenging properties	Filina et al (1995) <sup>75</sup>	+		
Alpha lipoic acid		Spare vitamins C and E & increases GSH levels				+
Vitamin B12 (cobalamin)		Free radical scavenging properties	Kishimoto M et al (1965) <sup>162</sup>			+
Ubiquinone (Coenzyme Q 10)		Free radical scavenging properties	Migliore L et al (2004) <sup>199</sup>			+
Melatonin	N-acetyl-5-methoxytryptamine	Free radical scavenging properties	Bandyopadhyay et al (2000) <sup>19</sup>	+		

+ = present in study.

GSH donate an electron to another molecule, a sulphur-centred thiyl radical, GS· is formed. When two such thiyl radicals (or two GSH molecules) join together the sulfhydryl (-SH) groups of cysteine are oxidized to form a disulphide bridge (-S-S-), as seen in the oxidized form of glutathione (GSSG) (Fig. 12B).<sup>220</sup> The glutathione status of a cell,<sup>168</sup> or the ratio of reduced to the oxidized form (GSH/GSSG), is an indicator of cellular redox imbalance or oxidative stress.<sup>23</sup>

### Heat Shock Proteins

Cells respond to various stressors by altering their gene transcription.<sup>169</sup> When cells are subjected to oxidative stress levels of various proteins may rise and simultaneously the levels of other proteins may

decrease. Independent of the type of stress induced, such as elevated temperatures, heavy metal exposure or oxidative stress, cells produce heat shock proteins.<sup>55</sup> The biological role of heat shock proteins is to function as molecular chaperones regulating the function of other proteins by binding to them, and modulating their function, transport, and folding state.<sup>176</sup>

### Transferrins

Transferrins play a role in antioxidant defense by binding free iron or metal ions in forms that will not stimulate free radical reactions.<sup>112</sup> Transition metals (e.g., copper, iron) are essential in most biological reactions, such as the synthesis of DNA, RNA, or proteins or as cofactors of numerous enzymes. A



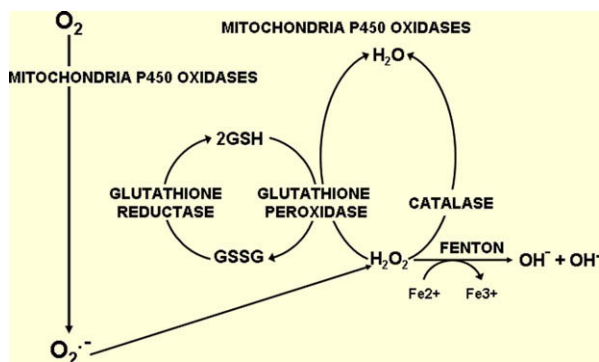


Fig. 11. Examples of enzymatic antioxidants and their reactions. Mitochondrial P450 oxidases can oxidize the oxygen molecule to the superoxide anion ( $O_2^{\bullet -}$ ). Glutathione reductase is an enzyme which reduces oxidized glutathione (glutathione disulphide, GSSG) to the reduced sulphhydryl form of glutathione (GSH). Glutathione peroxidase is an enzyme with peroxidase activity, which reduces hydrogen peroxide ( $H_2O_2$ ) to water ( $H_2O$ ) by oxidizing glutathione (GSH). The enzyme catalase converts hydrogen peroxide ( $H_2O_2$ ) to water ( $H_2O$ ) and oxygen ( $O_2$ )

deficiency in these metals can therefore lead to disturbances of the central nervous system or other organ functions.<sup>146</sup> Still, the accumulation of redox-active transition metals in tissues in excess of the capacity of cellular proteins (catalytic, transport, storage) capable of binding to these metals, is cytotoxic. Iron, for example, as long as it is bound by proteins in the body, does not cause any harm; but, if left unbound, or free, it can induce free radical production.<sup>158,185,255,326</sup> The rust ring around a metallic corneal foreign body is an example of a free radical reaction. Transferrin therefore acts as an antioxidant defense by binding transition metal ions in forms that will not stimulate free radical reactions.<sup>112</sup>

### Haptoglobins

Heme and heme proteins can act as pro-oxidants when heme proteins are released from damaged cells such as lysed erythrocytes.<sup>18,100,305</sup> Heme is a complex of protoporphyrin IX with iron that is pentacoordinated and in the Fe(III) oxidation state.<sup>186</sup> Iron has the capacity to accept and donate electrons readily and to interconvert between ferric ( $Fe^{4+}$ ) and ferrous ( $Fe^{3+}$ ). Free ferrous iron has been suggested to catalyze tissue damage by reacting with hydrogen peroxide to generate the highly reactive hydroxyl radical.<sup>111</sup> In the ferric state of heme protein, iron can react with hydrogen peroxide in vivo to produce the ferryl iron ( $Fe^{5+}$ ) and a protein-bound free radical, both of which exert prooxidative activities (Fig. 12C).<sup>4,132,205,232,291</sup> Plasma contains hemeoglobin-binding proteins

known as haptoglobins, as well as a heme-binding protein or hemeopexin.<sup>40</sup> Hemeoglobin-binding proteins decrease the effectiveness of these compounds in stimulating lipid peroxidation.

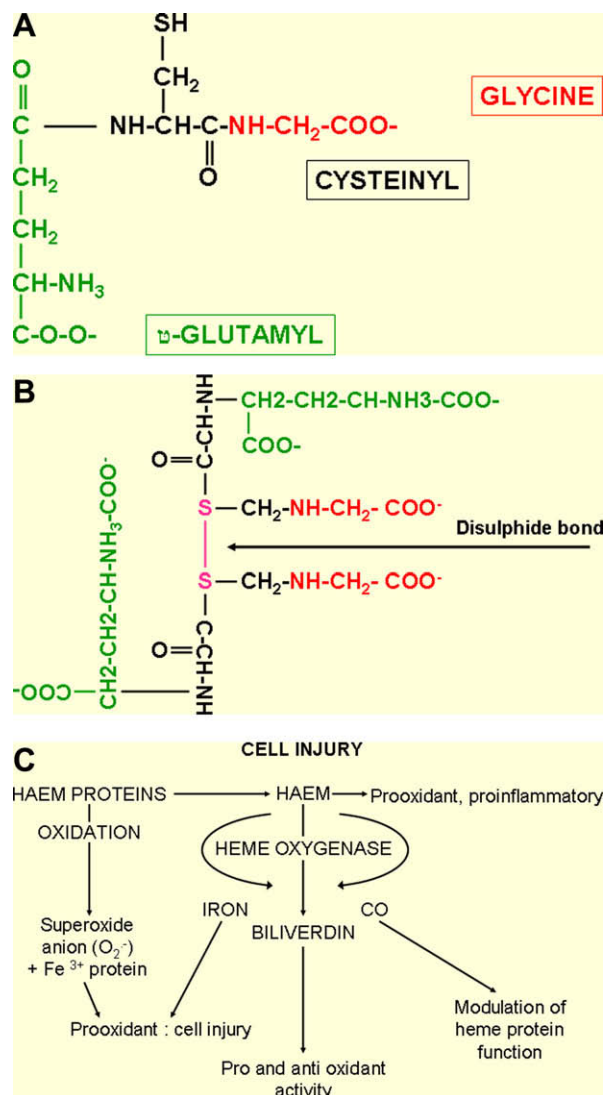
## Oxidative Stress

In the physiological state, the production of pro-oxidants is approximately balanced by the antioxidant defense system. The term *oxidative stress* is used to indicate when the intensity of pro-oxidants exceed the antioxidant capacity (Fig. 13A).<sup>265</sup> The generation of ROS and antioxidant mechanisms appear more or less balanced in healthy physiological state. In fact, the balance may be slightly tipped in favor of the ROS so that there is continuous low-level oxidative damage in the human body. This creates a need for antioxidant defense and repair.<sup>142</sup> The body can adapt to a certain degree of oxidative stress by increasing its defense mechanisms. If, however, the production of free radicals exceeds the antioxidative capacity, oxidative stress results.<sup>265</sup> Fortunately, nature can repair damage to a certain extent.<sup>49,252,260</sup> If the damage induced, however, is greater than the repair capacity, a disease can develop.<sup>49,145,252,260</sup> Oxidative stress induces damage to macromolecules and secondarily increases the levels of free calcium ion ( $Ca^{3+}$ ),<sup>216</sup> free intracellular iron (thereby inducing the hydroxyl radical),<sup>219</sup> and alters cellular signalling pathways.<sup>34,50,226</sup> These changes ultimately lead to additional damage of macromolecules (Fig. 13B).

## The Role of Oxidative Stress in the Pathophysiology of Glaucoma

### OXIDATIVE STRESS AND INTRAOCULAR PRESSURE ELEVATION

Oxidative stress has been implicated to be a cause of increased IOP by triggering trabecular meshwork degeneration and thus contributing to alterations in the aqueous outflow pathway.<sup>245,247</sup> The human trabecular meshwork is composed of collagen lamellae lined by endothelial cells. The space between the collagen beams of the trabecular meshwork is filled with extracellular matrix, composed mostly of glycoproteins and proteoglycans, where the aqueous humor filters through.<sup>293,331</sup> The trabecular meshwork is in constant contact with the aqueous humor from which ROS may be generated through light catalyzed reactions, metabolic pathways, or inflammation.<sup>242,245,270,293</sup> Disturbance of the trabecular meshwork cell status by an insult such as oxidative stress may lead to cellular loss and an overexpression or alteration in the structures of



**Fig. 12.** A: Glutathione is a protein composed of the three amino acids cysteine, glutamic acid, and glycine. This protein has strong antioxidant capacity as it is found at a high concentration in the liver, the principal organ involved in the detoxification and elimination of toxic materials. B: In the oxidized form of glutathione (GSSG), two glutathione (GSH) molecules join together as the sulphhydryl (-SH) groups of cysteine are oxidized to form a disulphide bridge (-S-S-). C: Iron-derived reactive oxygen species (ROS) are implicated in the pathogenesis of numerous vascular disorders including reperfusion injury. One abundant source of redox-active iron is heme, which is inherently dangerous when released from intracellular heme proteins. The iron in the heme rings of hemoglobin is in the Fe<sup>3+</sup> and essentially remains so when oxygen (O<sub>2</sub>) binds to it. Every once in a while a molecule of oxyhemoglobin undergoes decomposition and releases a superoxide anion (O<sub>2</sub><sup>-</sup>), which may cause cell injury. Free heme also promotes the conversion of low-density lipoprotein to cytotoxic oxidized products. Free hemoglobin in plasma, when oxidized, can transfer heme to endothelium, thereby enhancing cellular susceptibility to oxidant-mediated injury. As a defence against such toxicity, upon exposure to free heme, endothelial cells up-regulate heme oxygenase-1 and ferritin. Heme oxy-

genase is a heme-degrading enzyme that opens the porphyrin ring, producing biliverdin, carbon monoxide and a free redox-active iron initiating further cellular injury.

various glycoproteins in the extracellular matrix,<sup>181,316,331</sup> which interfere with the trabecular meshwork function, and lead to impaired aqueous humor outflow and thereby an increase in IOP.

The pathogenic role of oxidative stress in increasing IOP by reducing aqueous outflow facility is supported by various experimental studies performed in vitro and in vivo. In vitro treatment of human trabecular meshwork cells with hydrogen peroxide alters cellular adhesion and integrity.<sup>331</sup> In an animal study, perfusion of trabecular meshwork cells with peroxide has shown to reduce aqueous humor drainage from the anterior chamber of the calf's eye.<sup>150</sup> In humans, oxidative DNA damage has been reported to be significantly higher in the trabecular meshwork cells of glaucoma patients than in those of age-matched controls.<sup>141</sup> Further studies demonstrate abundant oxidative nucleotide modification (8-OH-dG) levels in human trabecular meshwork to be significantly correlated to the increase in IOP and to visual field damage.<sup>150,248</sup> Further evidence suggests that patients with POAG exert mitochondrial abnormalities implicating that mitochondrial dysfunction is most probably a consequence of oxidative stress.<sup>2</sup> Free radicals, contained in the aqueous humor, contribute to pathogenic alterations in the trabecular meshwork.<sup>7</sup> Findings show resistance to the outflow of aqueous humor of calf, as a result of trabecular meshwork cytoskeletal rearrangements and cellular loss, in the presence of increased levels of hydrogen peroxide.<sup>150</sup> The damage done by pro-oxidants to the aqueous humor outflow system supports reports that radiologists more often suffer from ocular hypertension.<sup>258</sup> At the molecular level, human trabecular meshwork endothelium has been reported to be an enriched site of nitric oxide synthesis. Nitric oxide can interact with oxygen or metals, such as copper or iron, to modulate outflow resistance of the trabecular meshwork.<sup>113</sup>

Moreover, findings suggest activity of the antioxidant defense in the aqueous outflow system: glaucoma patients display a significant depletion of total antioxidant potential in their aqueous humor,<sup>74</sup> a decrease in plasmatic glutathione levels,<sup>97</sup> and an increase in serum antibodies against glutathione-S-transferase.<sup>322</sup> The expression of endothelial-leukocyte adhesion molecule (ELAM-1), which provides protection against oxidative stress, is increased in the trabecular meshwork of glaucoma patients.<sup>310</sup> Another notable antioxidant, glutathione, is also found

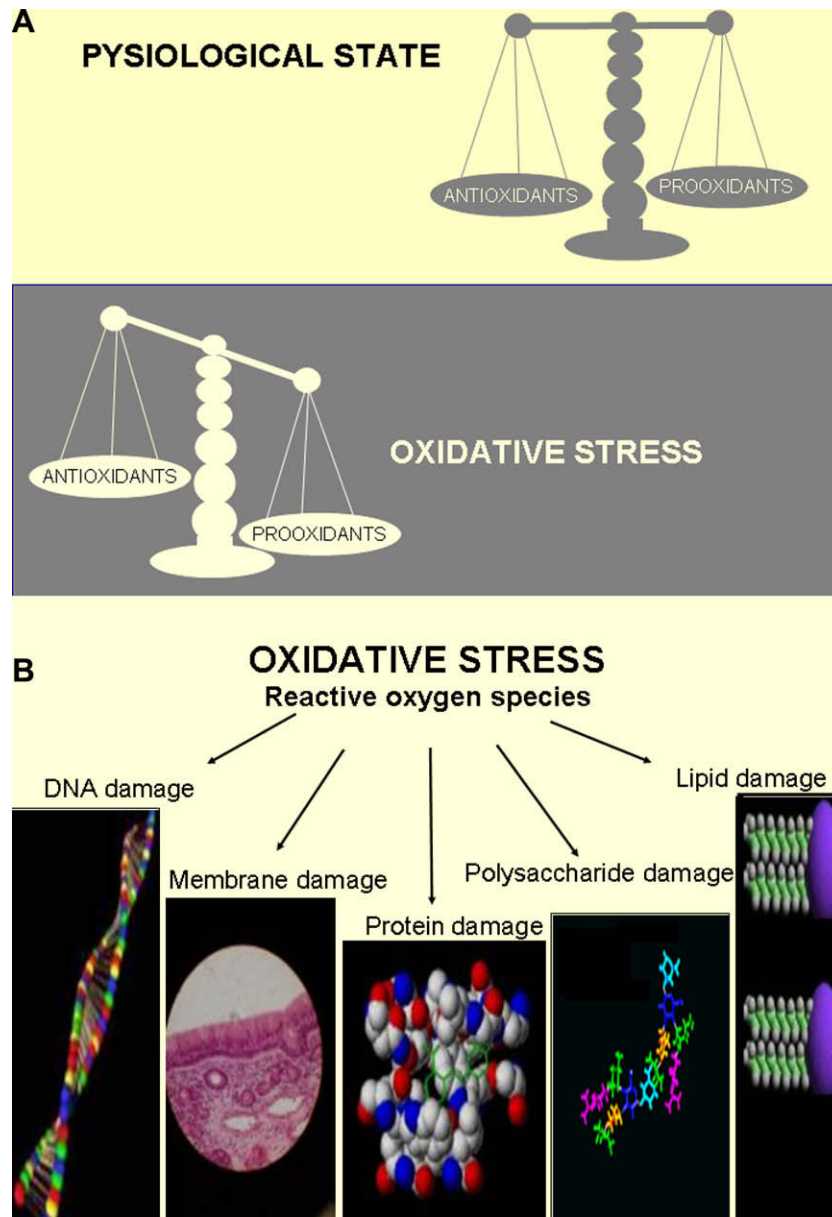


Fig. 13. A: In the physiological state, the production of pro-oxidants is approximately balanced by the antioxidant defense system. The term *oxidative stress* is referred to when the intensity of prooxidants exceed the antioxidant capacity. B: Reactive oxygen species (ROS) damage a variety of molecules such as DNA, membranes, proteins, polysaccharides, and lipids.

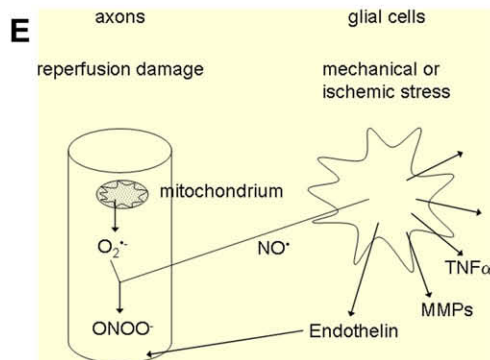
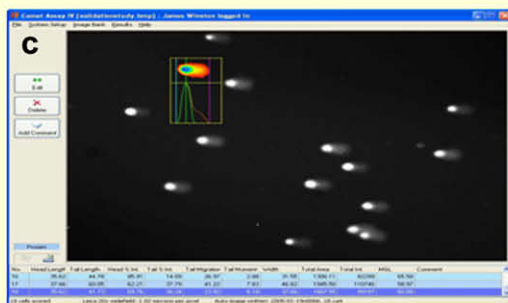
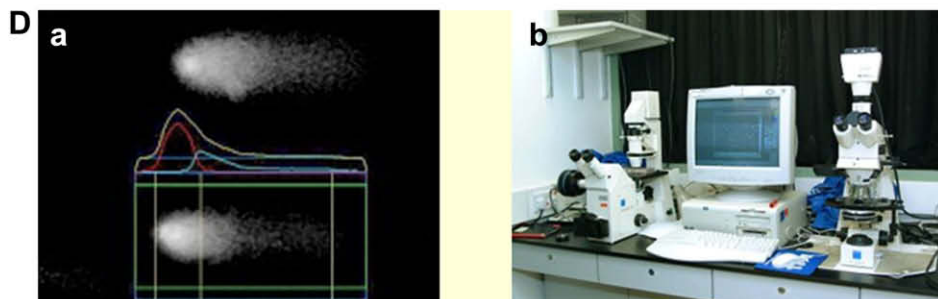
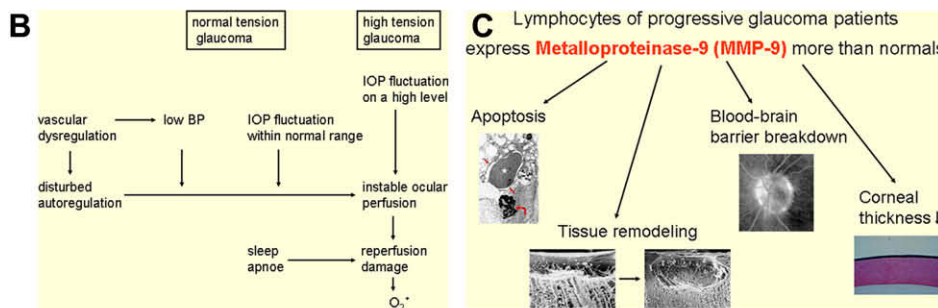
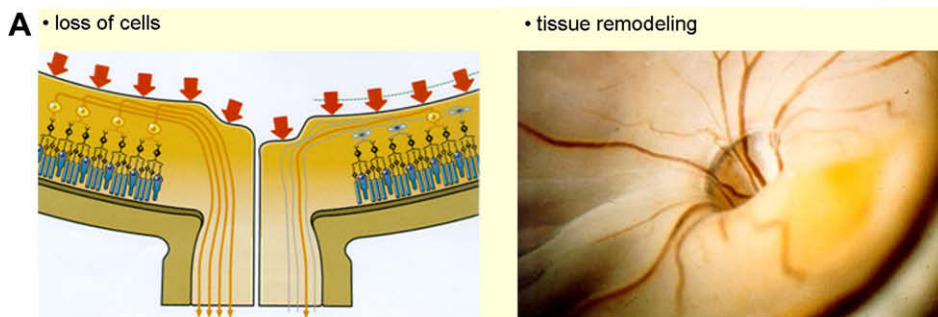
in high concentrations in both aqueous humor and in the trabecular meshwork of mammals.<sup>150,236</sup> In addition, the heat shock protein, alpha-B crystalline, which protects from oxidative damage, is overly expressed in trabecular meshwork cells of human and monkey eyes stressed by heat<sup>280,329</sup> and in the rat glaucoma model.<sup>224</sup> All these changes can be primary or secondary of nature.

**OXIDATIVE STRESS AND GLAUCOMATOUS OPTIC NEUROPATHY (GON)**

The pathomechanism leading to glaucomatous optic neuropathy (GON) is not yet fully un-

derstood.<sup>79</sup> Increased IOP is the most important risk factor and mechanical forces stretch the beams of the lamina cribrosa and distort the axons of the optic nerve head. Mechanical stress also leads to the activation of astrocytes via the stimulation of the epidermal growth factor receptor (Fig. 14A).<sup>184</sup>

The fact that, by far, not all glaucoma patients suffer from elevated IOP, along with the observation that the majority of glaucoma patients show signs of reduced ocular blood flow as well as ischemic signs in the eye (e.g., upregulation of the ischemia inducible factor 1- $\alpha$ ),<sup>286</sup> indicates that other



factors, in particular hemodynamic factors are involved as well.<sup>81</sup>

Ocular blood flow is, however, also reduced in a number of other diseases, as for example in multiple sclerosis<sup>222</sup> (due to a high level of circulating endothelin<sup>121</sup>), indicating that ischemia by itself does not induce GON. Even patients with a carotid stenosis do not suffer significantly more often from GON.<sup>225</sup>

Whereas on one hand, glaucoma patients have reduced ocular blood flow and this ocular blood flow reduction even has a predictive power for the progression of GON,<sup>253</sup> on the other hand, a blood flow reduction by atherosclerosis, multiple sclerosis, or other similar ischemic diseases, increases the risk for GON insignificantly. The solution of this seemingly paradox observation is simply the fact that it is not a stable reduction in ocular blood flow but rather the instability in blood flow that may lead to GON.<sup>81</sup> Such an instability in ocular blood flow leads to a repeated mild reperfusion injury (Fig. 14B).<sup>77</sup> This hypothesis is supported by the observation that IOP fluctuation is more damaging than a stable increase in IOP,<sup>10,78,217,313</sup> and by observations that patients that progress despite a normalized IOP suffer from a disturbed autoregulation.<sup>98</sup> The main cause for this insufficient autoregulation is a primary vascular dysregulation (PVD) syndrome.<sup>82</sup>

As described previously, oxidative stress occurs under a condition of high energy consumption, light exposure, or age-dependent decline of coping capacity to deal with free radicals. In glaucoma, an additional major factor is most likely a repeated mild reperfusion injury. This hypothesis is supported by observations on circulating lymphocytes of human glaucoma patients. Activated astrocytes express MHC-II capable of communicating with lymphocytes and lymphocytes also communicate with the capillary endothelial cells during reperfusion.<sup>164</sup> Indeed, the lymphocytes of glaucoma eyes express neural thread protein (NTP),<sup>101</sup> indicating axonal damage, but they also reveal upregulation of p53 and upregulation of proteasome 20 S subunits,<sup>318</sup> along with an over-expression of MMP-9 (Fig. 14C). Moreover, glaucoma patients exert an increase in lipid peroxidation products in their plasma in comparison to controls.<sup>71,324</sup> These re-

ports, together with observations of increased DNA breaks in circulating lymphocytes (Fig. 14D),<sup>203</sup> support the assumption of an increased oxidative stress in glaucoma patients.<sup>77</sup>

Although this might be a quite general aspect (these patients also suffer from silent myocardial ischemia),<sup>306</sup> the question arises as to why the eye and, in particular, the optic nerve head (ONH) gets damaged. In the ONH, blood flow is particularly unstable as a result of mechanical stress and an insufficient blood-brain barrier giving access of vasoactive substances to the pericytes and smooth muscle cells.<sup>131</sup> In addition, there is very high energy consumption in the axons of the optic nerve head due to the lack of myelin sheaths and therefore a high concentration of mitochondria in this area.

During reperfusion the main source of free radicals in cells lacking xanthine oxidase (all neural cells) are the mitochondria.<sup>8</sup> Whereas oxidative stress damages all types of molecules and thereby reduces the probability of cellular survival, the question arises as to why in glaucoma specifically the retinal ganglion cells and their axons die by apoptosis.

As mentioned previously, astrocytes activated by mechanical or ischemic stress produce a large amount of nitric oxide.<sup>214</sup> Nitric oxide, although having a very short half-life in biological tissues, has a small size and is liposoluble and can therefore readily diffuse to neighboring cells such as the axons of the ONH. If simultaneously, as a result of reperfusion, the concentration of superoxide ( $O_2^-$ ) is high, the very damaging peroxynitrite is produced (Fig. 14E).<sup>213</sup> Both superoxide anions and peroxynitrite are water soluble and can therefore not diffuse out of an intact cell membrane. They can, however, diffuse within the axons both toward the retina and the lateral geniculate nucleus.<sup>80,187</sup> In fact, nitrosylation of SH groups have been found in the retina and in the lateral geniculate nucleus implying the presence of peroxynitrite.

Reduced oxygen supply, itself, induces only mild oxidative stress. A marked reduction in oxygen supply to tissues could lead to an infarct. This can also occur in the eye (including the ONH), as well as in any other organ. A fluctuating circulation leads to an unstable oxygen supply. This, in turn, leads to

Fig. 14. A: Comet assay analysis of DNA breaks. a: The greater the amount of DNA breaks, the longer the comet tail; b: Microscope and software equipment; c: Most comet measurements provided by the image analysis software are very reliable. These measurements include percent DNA in the tail and the distribution of DNA in the tail. B: Glaucomatous optic neuropathy (GON) implies the loss of cells and tissue remodeling. C: Unstable ocular blood flow (OBF) leads to a repeated mild reperfusion and thereby the production of free radicals ( $O_2^-$ ) inducing injury. D: Metalloproteinase 9 (MMP9) is involved in tissue remodeling. E: The reaction between the superoxide anion ( $O_2^-$ ) produced by the mitochondria located numerously in the axons of the optic nerve head and nitric oxide ( $NO^*$ ) produces the highly damaging peroxynitrite ( $ONOO^*$ ).

a repeated mild reperfusion and thereby to injury by oxidative stress.<sup>1,190,307</sup> Reperfusion injury in glaucoma patients, particularly in the optic nerve head, is very mild but occurs repeatedly.<sup>77</sup> The assumption of reperfusion injury being involved in the pathogenesis also explains why sleep apnea<sup>204</sup> or reversible shock-like states<sup>57</sup> can lead to GON. ROS damage macromolecules such as proteins, lipids, or plasma membranes. The damage to the cells, in turn, causes the release of more free radicals. In prolonged ischemia hypoxanthin is formed as a result of the breakdown of ATP and the enzyme xanthine dehydrogenase is converted to xanthine oxidase. This also results in molecular oxygen being converted to the highly reactive superoxide and hydroxyl radicals, further resulting in tissue damage.<sup>110,153</sup> The nerve cells of CNS, however, lack xanthine oxidase (with the exception of blood vessels). In the CNS, ROS formation therefore does not occur via xanthine oxidase. The major source of oxidative stress in reperfusion stems from the mitochondria which are very crowded in the ONH due to high energy consumption in these nerve fibres lacking myelin sheaths.<sup>8</sup> The role of oxidative stress is further supported by findings of weaker antioxidant defense systems in POAG patients.

From a therapeutical view, the situation may be improved by stabilizing ocular blood flow (reducing and stabilizing IOP or increasing and stabilizing blood pressure or improving autoregulation) or treating with antioxidants as described subsequently.

## Natural Substances with Antioxidant Activity

### POLYPHENOLIC FLAVONOIDS

#### Tea

Green tea has the highest level of phenolic compounds among foods, about 35% by dry weight.<sup>196</sup> This tea is a rich source of flavonoids such as catechin (C), epicatechin (EC), and epigallocatechin (EGC) (Fig. 15A). The difference between black and green tea is how the leaf is processed after picking. In black tea, catechins are converted to complex fermentation products, namely theaflavins (TFs) and thearubigins (TGs), which give black tea its characteristic color.<sup>212</sup>

Tea flavonoids have been reported to have powerful antioxidative properties, as a result of their free radical scavenging properties. In fact, the polyphenolic compounds in tea have been shown to act as efficient scavengers for the superoxide anion<sup>264</sup> hydrogen peroxide and thereby partially inhibits ultraviolet-induced oxidative DNA damage.<sup>314</sup> Flavonoids present in green tea are able to inhibit the

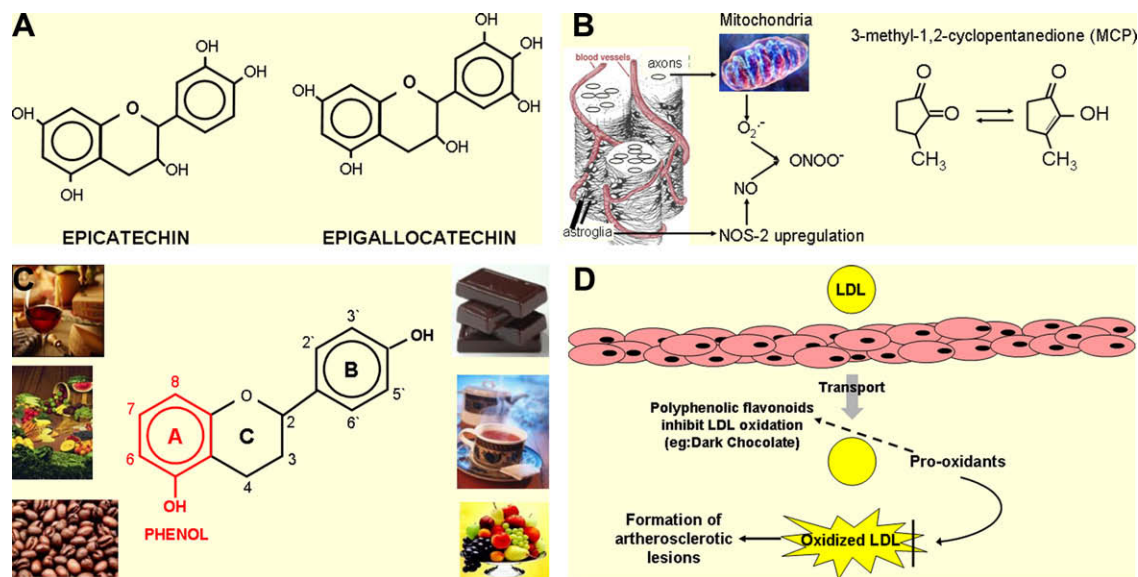
formation of lipid peroxy radical species and to act as inhibitors of low-density-lipoprotein (LDL) peroxidation.<sup>251</sup> Catechin, for example, has also been found to decrease iron-mediated lipid peroxidation and attenuate toxicity to iron-treated hepatocytes as well as to remove iron from iron-loaded hepatocytes.<sup>206</sup> Studies of the effect of polyphenols in green tea on cancer are controversial.<sup>96,133,159,277,311,321</sup> Due to their neuroprotective effects,<sup>191,234</sup> the use of green and black tea may prove to be of therapeutic value in the treatment of glaucoma.<sup>237</sup> In fact a new Italian product on market sells EGC (epigallocatechin) tablets for glaucoma under the generic name epinerve.

#### Coffee

Coffee beans contain about 8% phenolic compounds,<sup>45</sup> with an antioxidative effect due to their radical scavenging<sup>48</sup> and metal-chelating activities.<sup>279,315</sup> The compound 3-methyl-1,2-cyclopentanedione (MCP), isolated from the coffee extract, is in fact, a selective scavenger of peroxynitrite (ONOO<sup>-</sup>) (Fig. 15B).<sup>160</sup> The chemistry of MCP enables it to easily donate a proton to peroxynitrite in order to neutralize it; this is brought about by the chemical conversion of one of its carbonyl groups, which becomes reduced to a hydroxyl group. Polyphenols in coffee have been shown to have an *in vitro* antioxidative activity by inhibiting lipid peroxidation (e.g., caffeic acid),<sup>211</sup> and exerting a protective effect against mutagenicity.<sup>273</sup> Currently, there is much debate on the beneficial effects of coffee for glaucoma. Some researchers provide evidence for a positive association between coffee consumption and elevated IOP;<sup>15,33,127</sup> larger amounts of fluid drunk within a short period of time can elevate IOP. Over the long term, however, coffee exerts beneficial antioxidant effects<sup>88,102,134,315,323</sup> and thereby neuroprotective<sup>35,243</sup> properties; its potential as natural therapy deserves more attention.

#### Wine

The concentration of polyphenolic flavonoids in an average red table wine are approximately 120 mg/l anthocyanins, 50 mg/l flavonols, 250 mg/l catechins, and 750 mg/l anthocyanogenic tannins (Fig. 15C).<sup>91</sup> Red wines exhibit a stronger antioxidant capacity than white wines due to their phenolic content.<sup>87,235</sup> Polyphenolic flavonoids in wine have been reported to improve endothelial dysfunction and lower the susceptibility of LDL lipids to oxidation.<sup>47</sup> An impairment in endothelial function may lead to damage to vascular cells and the surrounding tissue. Endothelial dysfunction,



*Fig. 15.* A: Epicatechin and Epigallocatechin are polyphenolic compounds containing more than one phenol ring. Phenol rings induce good antioxidant capacity to the molecule, as a proton can readily be donated to neutralize free radicals. B: The compound 3-methyl-1, 2-cyclopentanedione (MCP) in coffee beans is a selective scavenger of the peroxynitrite. The chemistry of MCP enables it to easily donate a proton to peroxynitrite in order to neutralize it; this is brought about by the chemical conversion of one of its carbonyl groups which becomes reduced to a hydroxyl group. C: General chemical structure of polyphenolic flavonoids, which are compounds containing more than one phenolic ring. D: Pro-oxidant oxidation of LDL and antioxidant defense.

therefore, plays a role in the pathogenesis of a variety of disorders, including glaucoma<sup>44,105</sup> and cardiovascular diseases.<sup>192</sup> Indeed, red wines strongly inhibit the synthesis of endothelin-1,<sup>46,120</sup> a vasoactive peptide that plays a crucial role in the pathogenesis of glaucoma.<sup>64</sup> Animal studies have also shown that polyphenolic flavonoids in wine potentially prevent the initiation of atherosclerotic plaque development.<sup>13,86,267,275</sup> Moreover, resveratrol, a polyphenol found in grapes and wine, has been shown to reduce extracellular levels of vascular endothelial growth factor (VEGF).<sup>94</sup> The mechanisms with which polyphenols affect endothelial function is due to their ability to stimulate the production of endothelial nitric oxide synthase (eNOS) and promote the production of nitric oxide, which induces vasodilation.<sup>175</sup> Red wine polyphenolic flavonoids are naturally available and have several biological actions, which make this drink a potentially important agent in the treatment of glaucoma.

### Dark Chocolate

A subclass of flavonoids, namely, flavan-3-ols and their oligomers (procyanidins), are constituents of cocoa beans from the seed of theobroma cacao.<sup>122,201</sup> Dark chocolate generally contains at least twice as much cacao,<sup>303</sup> and therefore twice as much polyphenol in comparison to milk chocolate; in addition, the milk in milk chocolates reduces the

effective resorption of cacao. The antioxidative capacity of cacao has been demonstrated to be higher than that of wine or green tea due to much higher levels of phenolic phytochemicals.<sup>174</sup> Various in vivo studies have provided support that the consumption of cacao-rich food, such as dark chocolate, is associated with a reduced risk for vascular disease.<sup>91,103,302</sup> The mechanism is due to the action of flavan-3-ols, which augment endothelial NOS, and thereby nitric oxide, to improve endothelium-dependant vasorelaxation.<sup>66,123,124,154</sup> Ingestion of cacao decreases both systolic and diastolic blood pressure,<sup>103,285</sup> improves insulin sensitivity,<sup>103</sup> reduces LDL oxidation susceptibility (thereby increasing the serum total antioxidant capacity and HDL-cholesterol concentrations<sup>308</sup>), and reduces blood platelet stickiness and clotting (Fig. 15D).<sup>137</sup> Due to its multiple beneficial effects dark chocolate could prove to be of value in the treatment of glaucoma.

### Ginkgo Bilboa

The ginkgo tree, ginkgo biloba, is indigenous to China, Japan, and Korea, where it exists in remote mountainous areas. The medicinal components of ginkgo are the leaves and the seeds of the ginkgo fruit, which contain flavonoids and terpenoids.<sup>197</sup> The polyphenolic flavonoids confer antioxidant properties to the extract,<sup>167,193</sup> whereas the terpenoids

act as antagonists of platelet-activating factor.<sup>25</sup> The antioxidative properties of ginkgo are due to its direct radical scavenging activity.<sup>274</sup> Ginkgo biloba prevents oxidative damage to mitochondria,<sup>60,61</sup> exhibits neuroprotective properties,<sup>129</sup> inhibits LDL oxidation,<sup>320</sup> has a relaxing effect on vascular walls,<sup>254</sup> and an antagonistic action on platelet activating factor.<sup>163</sup> The effective radical scavenging property of ginkgo allows protection against retinal ischemia-reperfusion injury.<sup>32,172,259</sup> Administration of ginkgo increases ocular blood flow velocity in patients,<sup>43</sup> and improves visual field in normal-tension glaucoma patients.<sup>229</sup> The numerous beneficial properties of ginkgo biloba suggest it to be of major therapeutic value in the treatment of glaucoma.<sup>238</sup> Efficacy and safety reports have suggested a daily dose of 120 mg to be sufficient and acceptable.<sup>229</sup>

## ANTHOCYANOSIDES

### Vaccinium Myrtillus (Bilberry)

The antioxidant properties of vaccinium myrtillus arises from its high levels of anthocyanosides. The particular chemistry of anthocyanosides, with a positively charged oxygen atom in the anthocyanin molecule, enables it to readily scavenge electrons. This explains why vaccinium myrtillus exerts free radical scavenging activity,<sup>17,189</sup> and thereby neuroprotective properties (Fig. 16).<sup>147,165</sup> The neuroprotective properties of bilberry arise from anthocyanosides, which have been shown to be beneficial in reversing the course of neuronal aging in animals.<sup>165</sup> Animal studies report that bilberry supplementation is effective in reversing the delirious effects of neurodegeneration by affecting calcium homeostasis and additionally improving

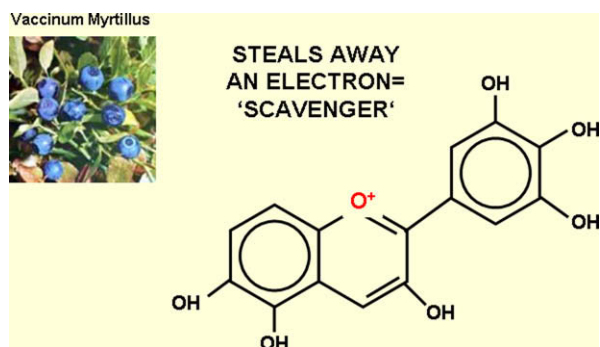


Fig. 16. Chemical structure of anthocyanin not only contains pheolic rings but also consists of a central ring with a positively charged oxygen atom. This positively charged oxygen atom can readily steal away or 'scavenge' electrons. This chemical structure gives foods containing anthocyanosides, such as bilberry, strong antioxidant properties.

motor performance.<sup>173</sup> The free radical scavenging property of bilberry was demonstrated in an assay of oxygen radical absorbance capacity (ORAC), where it was found to have highest ORAC scores among 30 fruits and vegetables tested.<sup>17</sup> Unfortunately studies analyzing the effect of bilberry on glaucoma are lacking; however, bilberry remains a candidate that deserves more intensive scrutiny.

## VITAMINS

### Alpha Lipoic Acid (ALA)

The water and fat soluble vitamin ALA is found in foods such as red meat, liver, and yeast. ALA is capable of regenerating several other antioxidants back to their active states, including vitamin C,<sup>188</sup> vitamin E,<sup>257</sup> glutathione,<sup>26</sup> and coenzyme Q10.<sup>149</sup> Preliminary evidence indicates that 150 mg of alpha lipoic acid, taken daily for 1 month, improves visual function in people with glaucoma.<sup>75</sup> Further research is warranted.

### Thiamin (Vitamin B1)

Levels of thiamin have been found to be lower in some glaucoma patients in comparison to controls, and a deficient absorption of this vitamin in these patients has been postulated.<sup>11</sup> Thiamine deficiency has been reported to cause neurodegeneration by inducing diverse changes in microglia,<sup>288</sup> astrocytes,<sup>312</sup> endothelial cells,<sup>29</sup> and mast cells,<sup>73</sup> leading to neuronal cell death. The brains of thiamine-deficient mice show vascular changes, inflammatory responses, and oxidative stress,<sup>29</sup> similar to the brains from patients who die from common neurodegenerative diseases.<sup>5,210</sup>

### Cobalamin (Vitamin B12)

No deficiency of cobalamin has been reported in glaucoma patients. However, when glaucoma patients have been supplemented with daily vitamin B12, no progression of visual field loss was observed during 1 year of follow-up.<sup>162</sup> The neuroprotective effect of vitamin B12<sup>183</sup> is likely due to its radical scavenging properties.<sup>294,296</sup> The possibility of neuroprotective activity in glaucoma that may be provided by natural nutrients, such as beef, liver, or salmon containing vitamin B12, is conceivable and deserves further investigation.<sup>239</sup>

## MISCELLANEOUS

### Ubiquinone (Coenzyme Q10)

Coenzyme Q10 is a coenzyme for the inner mitochondrial enzyme complexes involved in energy production within the cell (Fig. 17). Coenzyme Q10 has been demonstrated to prevent lipid



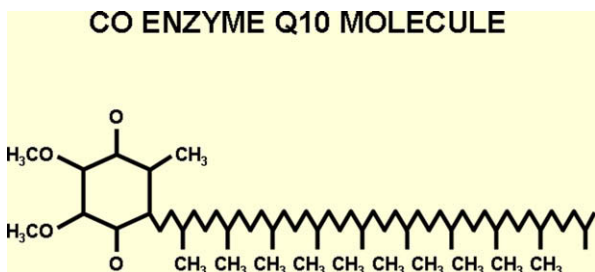


Fig. 17. Chemical structure of the co-enzyme Q10 molecule.

peroxidation and DNA damage induced by oxidative stress.<sup>41,289</sup> In vivo supplementation with co-enzyme Q10 enhances the recovery of human lymphocytes from oxidative DNA damage.<sup>199</sup> These properties are due to ubiquinones free radical scavenging activity.<sup>89</sup> In one study of glaucoma patients, administration of oral ubiquinone was shown to be useful in mitigating cardiovascular side-effects without affecting IOP.<sup>278</sup> Unfortunately studies examining the effect of ubiquinone on glaucoma are lacking and therefore currently limit the use of this agent.

### Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine, secreted by the pineal gland, which has a regulatory role in circadian rhythms in mammals (Fig. 18). Various reports suggest that

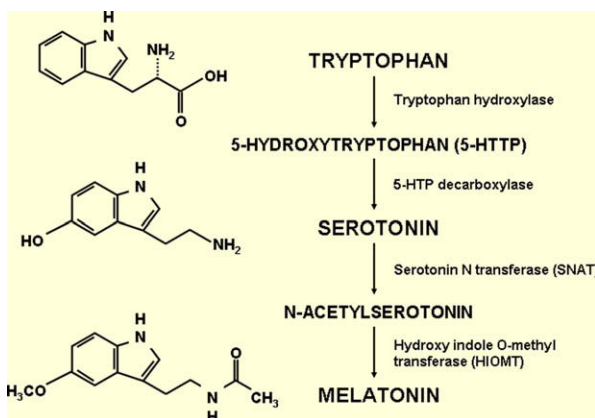


Fig. 18. Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine, secreted by the pineal gland, which has a regulatory role in circadian rhythms in mammals. Melatonin is naturally synthesized from the amino acid tryptophan via synthesis of serotonin by the enzyme 5-hydroxyindole-O-methyltransferase. The production of melatonin is primarily nocturnal and is controlled by the exposure to cycles of light and dark, independent of sleep. Melatonin is a potent free-radical scavenger, and because melatonin production decreases with age, might also be an important factor in the aging process.

melatonin exerts antioxidant activity. Melatonin has been shown to detoxify directly the highly damaging hydroxyl radical ( $\text{OH}\cdot$ );<sup>19,281</sup> moreover, reports suggest the ability of melatonin to neutralize a variety of other pro-oxidants, including hydrogen peroxide, singlet oxygen, nitric oxide, and the highly damaging peroxy nitrite anion.<sup>6,16,20,37,92,283</sup> In the retina, melatonin reduces the elevation of cGMP by suppressing NOS activity, indicating a neuroprotective role.<sup>249</sup>

Findings indicate that melatonin reduces nitric oxide-induced retinal oxidative damage both in vitro and in vivo.<sup>268</sup> Furthermore, several of the metabolites that are generated when melatonin inactivates toxic reactants are themselves free radical scavengers.<sup>107,119,221,266</sup> In addition, melatonin stimulates a number of antioxidative enzymes, which further promote antioxidative protection.<sup>233,241</sup> At the mitochondrial level, melatonin has been shown to reduce electron leakage from respiratory complexes and scavenge free radicals.<sup>177,178</sup> A vast number of findings also suggest that melatonin can protect against reperfusion/ischemia injury.<sup>53,54,68,109,208,250,282</sup> Studies on the effect of melatonin on glaucoma are limited.<sup>38</sup> In light of its antioxidative and neuroprotective properties,<sup>39,261,272,309,325</sup> the potential therapeutic use of melatonin for glaucoma warrants considerate evaluation.

### Potential Value of Antioxidants for Glaucoma

There is still much debate about the value of natural antioxidant or vitamin supplementation on ocular diseases such as glaucoma.<sup>21,298,332</sup> Because the pathogenesis of glaucoma involves various factors, one of which may be oxidative stress, the possibility that natural dietary antioxidants or vitamin supplementations may be beneficial becomes plausible. If diet proves to be beneficial it will be one of the most cost-effective treatments for glaucoma, the incidence of which is expected to increase by 2030, mostly because of the aging population.<sup>230,290</sup> This disease may in fact be an even more relevant problem in developing countries with social or economical problems. Unfortunately, however, the effect of diet cannot be easily measured due to confounding factors such as timing of diet exposure, level of diet, or intake of other nutrients.<sup>126</sup>

A number of studies deal with the antioxidant role of polyphenolic compounds in our health. Still, study results are difficult to evaluate and interpret because of the large number of phenolic compounds and the fact that the phenolic content of

most foods is not well established. Although there have been major research advancements in the identification and characterization of specific polyphenols,<sup>69,76,84,194</sup> many remain unidentified. To illustrate these difficulties, we would like to refer to the question whether polyphenols in tea have an effect for cancer prevention. Whereas certain studies reported a significant improvement between green tea consumption and cancer risk,<sup>108,156,262,328</sup> two others<sup>133,170</sup> along with a large meta-analysis of epidemiological studies<sup>277</sup> found no such association. This may be a problem of methodology. A close scrutiny of the polyphenolic studies shows that most were in vitro assessments,<sup>3,108,234,251,314</sup> and only a few are done in vivo. Moreover, the bioavailability or delivery of polyphenols to a specific tissue site is also often not accounted for. Although results of in vitro assessments may be very promising, they do not necessarily reflect in vivo changes. The functional significance of the reports is therefore often not clearly established.

Still, our enthusiasm in research of polyphenols should not be diminished. Both in vitro and in vivo studies done on ginkgo biloba, rich in both polyphenols and terpenoid compounds, are very promising. An in vitro study reports ginkgo to stabilize and protect mitochondrial function.<sup>61</sup> In addition, reports suggest ginkgo to have neuroprotective effects on retinal ganglion cells in a rat model,<sup>129</sup> and to improve pre-existing visual field damage in normal tension glaucoma patients.<sup>229</sup> This may partially be due to the fact that in contrast to other antioxidants (vitamin E, vitamin C), ginkgo has the capacity to enter the inner mitochondrial membrane, making it a particularly effective antioxidant at mitochondrial level. The impact of the beneficial effects of ginkgo will be even greater if future studies support these results. In the meantime, even if future studies do not support this evidence we have done our patients no disservice by treating with ginkgo extracts when keeping possible contraindications in perspective (e.g., should not be given to patients on anticoagulants).

We encounter similar problems when considering the therapeutic value of anthocyanosides. In the 1960s it was suggested that anthocyanoside extracts in *V. myrtillus* improve human vision in reduced light conditions.<sup>30</sup> More recent studies, however, have failed to find such an effect.<sup>180,209,327</sup> These outcomes may be confounded by various factors including dose, choice of subject, or possible geographical variations in extract composition. Still, the fact that we cannot conclusively verify beneficial antioxidant effects of anthocyanosides in *V. myrtillus* does not necessarily mean that no beneficial effects exist. We are confronted with a scientific challenge that is more

difficult to solve. The fact remains that there are multiple plausible mechanisms by which anthocyanosides in *V. myrtillus* could exert beneficial effects. We can therefore only conclude that the potential beneficial effects of this extract remains to be seen. Moreover, there seems to be no reason to withhold natural food sources of anthocyanosides from our patients, especially if side effects are rare.

Similarly, the potential protective effect of vitamin supplementation has not been thoroughly evaluated. Among the various reports concerning the beneficial effects of vitamin supplementation to promote anti-oxidation, other reports clearly underline the adverse effects of supplementation. This is not surprising. Because physiological systems of antioxidant defence are complex it makes sense that optimal functioning requires the availability of numerous antioxidants. Foods provide an even greater array of antioxidants than supplements and a correctly dosed combination of antioxidants, as found naturally, in foods, appears to be more effective.

The best possible example comes from observations of results of various epidemiological and clinical studies done on vitamin C (water soluble) and vitamin E (lipid soluble) supplementation. The adverse pro-oxidant effects of these two vitamins have clearly been underlined in various studies: a meta-analysis of 19 randomized, controlled trials including more than 135,000 participants found that high-dosed vitamin E supplementation ( $\geq 400$  IU/day for at least 1 year) increased mortality;<sup>200</sup> similarly, vitamin C supplementation (500 mg/day for 6 weeks) administered as a dietary supplement to healthy humans was shown to exhibit a pro-oxidant effect.<sup>223</sup> In fact, these two vitamins have highest antioxidant capacity when working synergistically together.<sup>36,99,148</sup> Vitamin C is capable of donating electrons to and reconverting vitamin E back to its active reduced state. Oxidized vitamin C, in turn,

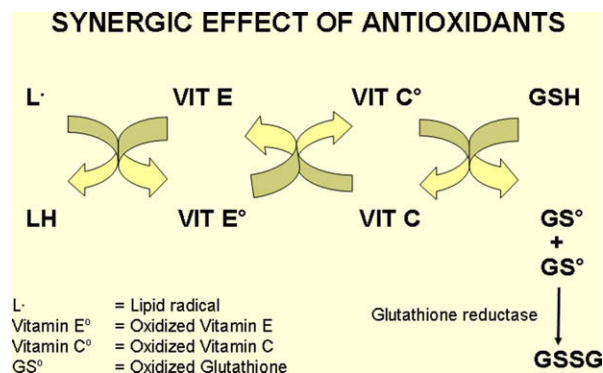


Fig. 19. Vitamin E can neutralize a free lipid radical. Vitamin C can, in turn, donate electrons to vitamin E and convert it into its reduced state. Oxidized vitamin C can then be reduced by the more potent antioxidant glutathione.

can be reduced after reacting with glutathione, or with the more potent antioxidant, alpha lipoic acid (Fig. 19). We can deduce the following from these observations: antioxidant supplementation may induce harmful pro-oxidant effects; antioxidant vitamins have the highest antioxidant capacity when they work synergistically; the synergic interaction between water and lipid soluble vitamins, overcomes the problem of work sites, as vitamins with different solubilities will exert effects at different cell sites (e.g., vitamin E cannot penetrate the mitochondria).

There is realistic hope that in the future we will be able to give more specific advice concerning diet to our patients. In the meantime, current evidence of nutritional value is sufficient for us to recommend our patients to consume foods and beverages rich in antioxidants.

### Conclusions

In POAG we still do not know the exact mechanisms that lead to damage of the trabecular meshwork and thereby to an increase in IOP, nor do we know the exact mechanisms leading to glaucomatous optic neuropathy. Obviously there are a number of factors and mechanisms involved. One of these factors is oxidative stress. At present, we only have a vague idea how oxidative stress is brought about in glaucoma and how it damages the tissue. Preliminary evidence, however, has already led to investigations of numerous compounds with antioxidant potential. Unfortunately, at this time we still do not conclusively know whether such a treatment can avoid damage or progression of damage. Growing evidence suggests, however, that oxidative stress is not just a minor side-pathway, but rather a crucial aspect in the pathogenesis of glaucoma. It is therefore worthwhile to summarize the present knowledge. Further investigations of the role of oxidative stress in the pathogenesis of glaucoma and the influence of antioxidant treatment seem to be rewarding.

### Method of Literature Search

A systematic search of the Medline database using Pubmed Web site for the years 1970 through February 2007, was conducted using the following key words: *POAG, oxidative stress, reactive oxygen species, singlet oxygen, superoxide anion, hydrogen peroxide, nitric oxide, peroxynitrite, antioxidant, superoxide dismutase, catalase, glutathione peroxidase, glutathione, heat shock protein, transferrin, haptoglobin, trabecular meshwork, intraocular pressure, outflow facility, glaucomatous optic neuropathy, ocular blood flow, vascular dysregulation, autoregulation, reperfusion injury, mitochondria, polyphenolic flavonoids,*

*gingko biloba, anthocyanosides, alpha lipoic acid, thiamine, cobalamin, ubiquinone, melatonin.* The old Medline was searched for articles published between 1960 and 1970 using the same key words. All articles read were in English and German, and when articles in other languages were deemed to be of relevance, their abstracts in English were read. Articles in Russian were translated if the abstract did not provide sufficient information.

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This article is dedicated to the memory of Bernard Schwartz, MD, PhD.

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