Antioxidant Defense: Vitamins E and C and Carotenoids

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Reactive oxygen species are thought to be implicated in the pathogenesis of various human diseases. They are generated endogenously under physiological and pathological conditions but also upon exposure to exogenous challenge. The organism maintains defense systems against reactive oxygen species, including enzymes and low-molecular-weight antioxidants. Important antioxidants such as vitamins E and C and carotenoids are provided from the diet. Vitamin E, as the major chain-breaking antioxidant, inhibits lipid peroxidation, thus preventing membrane damage and modification of low-density lipoproteins. It is regenerated by the water-soluble vitamin C. Carotenoids efficiently scavenge singlet molecular oxygen and peroxyl radicals. There is increasing evidence from epidemiological studies, animal experiments, and in vitro investigations that an increased intake of antioxidants is associated with a diminished risk for several diseases. *Diabetes* **46 (Suppl. 2):S14–S18, 1997**

FORMATION AND CHEMICAL PROPERTIES OF REACTIVE OXYGEN SPECIES

The generation of reactive oxygen species is associated with life in aerobic conditions, and reactive intermediates are produced under physiological and pathophysiological conditions (1). Reactive oxygen species are capable of damaging biological macromolecules such as DNA, carbohydrates, or proteins. These damaging processes are currently being discussed as pathobiochemical mechanisms involved in the initiation or progression of various diseases (2,3).

Some of the most reactive oxygen species and their estimated half-lives in biological systems are listed in Table 1. The species are either radicals (molecules that contain at least one unpaired electron), such as hydroxyl radical and peroxyl radical, or reactive nonradical compounds, such as singlet oxygen, peroxynitrite, and hydrogen peroxide. The half-lives vary from a few nanoseconds for the most reactive compounds to seconds or hours for rather stable radicals. Hydrogen peroxide can be stored indefinitely under suitable conditions.

Various sources of reactive oxygen species have been identified in living organisms (4–6). The superoxide radical anion appears to play a central role, since other reactive intermediates are formed from it (Fig.1). Superoxide is formed by one-electron reduction of oxygen mediated by enzymes such as NADPH oxidase or xanthine oxidase or by the respiratory chain. It has been estimated that leakage of the respiratory chain may account for 1–3% of the oxygen utilized for energy production, thus amounting to a high potential burden of aerobic organisms. The half-life of O_2^- in tissues depends on the presence of the enzyme superoxide dismutase in different compartments. Reactive oxygen species are also produced in the organism as a part of the primary immune defense. Phagocytic cells such as neutrophils, monocytes, or macrophages defend against foreign organisms by generating O_2^- and nitric oxide as a part of the killing mechanism (7). Both compounds can combine (Fig.1) to peroxynitrite ($ONOO^-$), a reactive species capable of inducing lipid peroxidation in lipoproteins. By impairing phosphorylation, peroxynitrite can interfere with cellular signaling by nitrating tyrosine residues in proteins (8).

Hydrogen peroxide (H_2O_2) can diffuse readily between cells. It is efficiently converted to water by the enzyme catalase or by glutathione peroxidase, a process that determines its half-life. Recent evidence suggests that hydrogen peroxide is involved in signal transduction, modulating the expression of genes through the NF-κB and AP-1 pathways (9–11).

The hydroxyl radical is the most reactive oxygen species, with an estimated half-life of a few nanoseconds. It is formed in vivo upon high energy irradiation (e.g., X rays), by homolytic cleavage of water, or from hydrogen peroxide in a metal-catalyzed process. Ultraviolet-light energy is insufficient to split water, but it can cleave hydrogen peroxide to yield the hydroxyl radical. Due to its high reactivity, this radical immediately reacts with surrounding target molecules at the site where it is generated.

Peroxyl radicals (ROO•) can be generated in the process of lipid peroxidation, which is initiated by the abstraction of an hydrogen atom from polyunsaturated fatty acids, e.g., the hydroxyl radical is capable of initiating this reaction sequence (12). ROO• are relatively long-lived species (seconds) with a considerable diffusion path length in biological systems.

Further products generated in lipid peroxidation are organic hydroperoxides (ROOH), which might rearrange to endoperoxide intermediates that are cleaved to yield aldehydes. The reaction of aldehydes with amine groups of peptides and proteins has been discussed as a mechanism involved in the modification of lipoproteins (6).

Singlet molecular oxygen ($^1O_2$) is electronically excited oxygen that is formed in biological systems via photosensitization reactions. This pathway is thought to be important in light-exposed tissue (13). Singlet oxygen might also be formed via chemi-excitation in reactions without activation by light. The half-life of singlet oxygen has been estimated as 10^{-6} to 10^{-5}

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TABLE 1
Estimated half-lives of reactive oxygen species

	Reactive oxygen species	Half-life
Radicals		
HO•	Hydroxyl radical	10^{-9}
RO•	Alkoxyl radical	10^{-6}
ROO•	Peroxyl radical	7
NO•	Nitric oxide radical	1–10
O_2^{-}•	Superoxide anion radical	Enzymic*
Non-radical species		
$^1O_2$	Singlet oxygen	10^{-5}
$ONOO^-$	Peroxynitrite	0.05–1
H_2O_2	Hydrogen peroxide	Enzymic*

*Scavenged by specific enzymes; half-lives depend on presence of these enzymes.

s. $^1O_2$ can interact with target molecules either by transferring its excitation energy or combining chemically. Preferential targets for chemical reactions are double bonds, e.g., in polyunsaturated fatty acids or guanine bases in DNA (2,13).

It should be noted that the organism is also exposed to reactive oxygen species from external sources. Many compounds of prooxidant nature, such as quinones capable of redox cycling, are delivered to the organism with the diet. Cigarette smoke contains an array of radicals that contribute to the increased risk of lung cancer among smokers. The toxicity of ozone, of which increasing levels are reported due to air pollution, is associated with oxidative attack against lung tissue.

ANTIOXIDANT DEFENSE

A variety of antioxidant defense systems are operative, including enzymatic and nonenzymatic antioxidants (14,15). Enzymes directly involved in the detoxification of reactive oxygen species are superoxide dismutase, catalase, and glutathione peroxidases. Indirect antioxidant functions are mediated by enzymes that restore endogenous antioxidant levels or proteins that control the levels of free iron or copper ions, thus preventing the formation of reactive oxygen species. Some endogenously synthesized low-molecular-weight compounds are also involved in antioxidant defense. Glutathione, the major cytosolic thiol, serves as a cofactor of several detoxifying enzymes and also scavenges reactive oxygen species being oxidized to GSSG.

An important source for antioxidants is diet, which contains numerous compounds exhibiting antioxidant activity (16–18). The most prominent dietary antioxidants are ascorbate (vitamin C), the tocopherols (vitamin E), and the carotenoids (Fig. 2); normal plasma levels are summarized in Table 2.

Vitamin C is one of the most powerful natural antioxidants (19). It is water soluble and found in high concentrations in many tissues. Human blood plasma contains about 60 μmol/l ascorbate, while about 15-fold higher levels are detected in tissues such as liver, kidney, pancreas, and thymus. Even higher levels are found in the eye lens and in adrenal tissue. Upon interaction with reactive oxygen species, vitamin C is oxidized to dehydroascorbate via the intermediate ascorbyl free radical. Dehydroascorbate is recycled back to ascorbic acid by dehydroascorbate reductase. Thus, dehydroascorbate occurs in very low levels compared with vitamin C. As a scavenger of reactive oxygen species, ascorbate has been shown to be effective against superoxide radical anion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen. In aqueous solutions, vitamin C also scavenges reactive nitrogen oxide species efficiently, preventing the nitrosation of target molecules. The major sources for ascorbate in the diet are fruits, especially citrus fruits, kiwi, cherries, and melons, and vegetables such as tomatoes, leafy greens, broccoli, cauliflower, Brussels sprouts, and cabbage; its content might exceed 100 mg/100 g wet weight. There is evidence from in vitro studies that vitamin C is capable of regenerating tocopherol from the tocopheroxyl radical that is formed upon inhibition of lipid peroxidation by vitamin E (20). It should be noted, however, that ascorbate can also act as prooxidant in vivo. In the presence of free transition metal

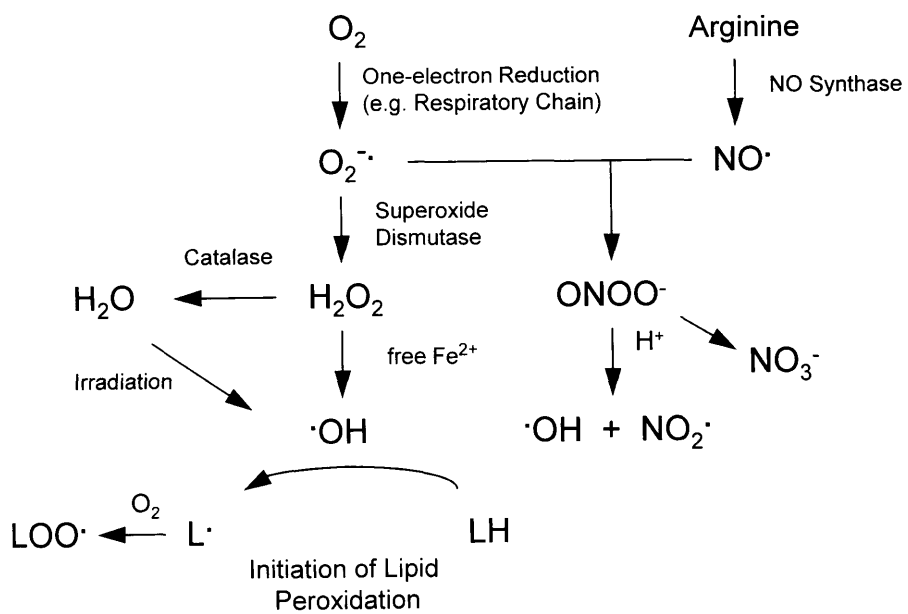
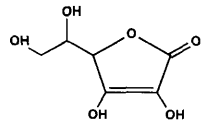
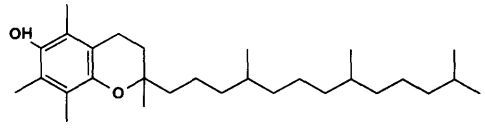


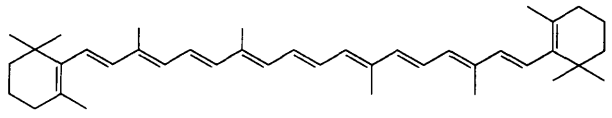
FIG. 1. Suggested pathways for the formation of reactive oxygen species in vivo.



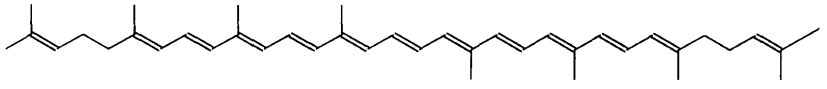
Vitamin C



Vitamin E



ß-Carotene



Lycopene

FIG. 2. Structures of vitamin C (ascorbate), vitamin E (α-tocopherol), β-carotene, and lycopene.

ions (iron and copper) and ascorbate, the hydroxyl radical can be generated, and initiation of lipid peroxidation may occur.

The term *vitamin E* is a generic description for all tocols and tocotrienol derivatives that exhibit the biological activity of α-tocopherol (21–23). This group of compounds is lipophilic, operative in membranes or lipoprotein particles. An important antioxidant function appears to be the inhibition of lipid peroxidation, scavenging lipid peroxyl radicals to yield lipid hydroperoxides and the tocopheroxyl radical. The latter is less reactive with neighboring polyunsaturated fatty acids than are peroxyl radicals and acts as a chain-breaking antioxidant. The tocopheroxyl radical can be either reduced by ascorbate or glutathione or further oxidized to the quinone. Since only small amounts of tocopheryl quinone are detectable in human blood and tissues, the regenerative pathway in vivo appears to be favored. Compared with other lipophilic antioxidants, α-tocopherol is probably the most efficient antioxidant in the lipid phase (24). It contains shielding methyl groups adjacent to the phenolic hydroxyl group, and it is optimally positioned in membranes by its phytyl side-chain. In addition to α-tocopherol's peroxyl radical scavenging properties, further interactions with reactive oxygen species have been described, including the quenching of singlet oxygen and the reaction with peroxynitrite. The richest sources for vitamin E in the diet are vegetable oils (soybean, corn, cottonseed, and safflower) and products made from these oils, such as margarine or mayonnaise. Further, wheat germ, nuts, and some green leafy vegetables contribute considerable amounts of vitamin E (21). Vitamin E plasma levels in the human are about 22 μmol/l. High levels are found in tissues such as liver, kidney, adrenals, and fatty tissue. In the liver, the RRR-isomer of α-tocopherol is preferentially incorporated into VLDLs, which are further catabolized in the cir-

TABLE 2
Antioxidant levels in human plasma

Antioxidant	Plasma level (μmol/l)
Water-soluble	
Ascorbate	30–150
Glutathione	1–5
Urate	160–450
Lipid-soluble	
β-carotene	0.3–0.6
Lycopene	0.5–1.0
Lutein	0.1–0.3
α-carotene	0.05–0.1
α-tocopherol	15–40
δ-tocopherol	3–5

culation. Thus, RRR-α-tocopherol is the major form of vitamin E in LDL (22).

Carotenoids are natural colorants with pronounced antioxidant activity (18,25). Their chemical properties are closely related to the presence of an extended system of conjugated double bonds that are substituted with various end groups. Reactive oxygen species that are efficiently scavenged by carotenoids are $^1O_2$ and peroxyl radicals. Two different pathways operate with respect to the deactivation of $^1O_2$: physical and chemical quenching. Physical quenching implies the deactivation of $^1O_2$ by energy transfer from the excited oxygen species to the carotenoid, yielding a triplet excited carotenoid. The energy of the excited carotenoid is dissipated through vibrational interactions with the solvent to recover the ground-state carotenoid. The carotenoid remains intact in this process and can undergo further cycles of deactivation. Chemical quenching contributes less than 0.05% to total $^1O_2$-quenching by carotenoids but is responsible for the eventual destruction of the molecule. Carotenoids are the most efficient naturally occurring quenchers for $^1O_2$, with quenching rate constants around $5–12 \times 10^9\ M^{-1} \cdot s^{-1}$. Carotenoids were reported to scavenge peroxyl radicals by chemical interaction. It is suggested that carotene radical intermediates are formed in this process, which finally leads to the destruction of the molecule. As does vitamin E, carotenoids belong to the group of lipophilic antioxidants present in lipoproteins such as LDL and HDL. It has been shown that carotenoids are consumed when isolated LDLs are exposed to conditions of lipid peroxidation. Their contribution to the antioxidant defense system of LDLs is not quite clear, since no regeneration pathways for oxidized carotenoids are known. A variety of structurally different carotenoids are present in fruits and vegetables. Some of the major sources are carrots (α-carotene, β-carotene), tomatoes (lycopene), citrus fruits (β-cryptoxanthin), spinach (lutein), and maize (zeaxanthin) (26).

OXIDATIVE DAMAGE AND DISEASE

Reactive oxygen species are suggested or known to be involved in the pathogenic processes of numerous diseases (12,27,28). There is increasing evidence from clinical and intervention studies, as well as from basic research, that antioxidants might prevent or delay the development of disease (29,30).

The primary cause for most cardiovascular diseases is thought to be arteriosclerosis. It is suggested that in early stages of arteriosclerosis, lipid deposits are formed in the subendothelial space, so-called fatty streaks. There is increasing evidence that an oxidative modification of LDL plays an important role in this pathogenic pathway. LDL oxidation is efficiently inhibited by lipophilic antioxidants such as α-tocopherol (12,14,27).

Patients with type 1 or type 2 diabetes have an increased risk for micro- and macrovascular diseases, such as retinopathy and nephropathy, and coronary heart diseases or diminished cerebral blood supply. These disease states are summarized as diabetic late syndrome and correlate with hyperglycemia as an essential etiologic factor. There is increasing evidence that an additional load of reactive oxygen species is formed in diabetes via glycoxidation, a process probably relevant at elevated glucose blood levels (31,32). A further pathway for the modification of LDL proteins involves the formation of Amadori products. These processes might explain an additional risk of LDL modification due to elevated glucose levels.

CONCLUSION

The imbalance between reactive oxygen species and antioxidant defense systems may increase the oxidative burden and lead to the damage of macromolecules. Such processes are thought to play a role in pathological processes of various diseases, including the diabetic late syndrome. A sufficient supply with antioxidants either from the diet or from supplements might help to prevent or delay the occurrence of these effects. However, further basic biochemical data and information from clinical studies are necessary to prove the benefits of antioxidant supplementation.

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