Current Topics in Pharmacol., 2 (1993)

Antioxidant vitamins and atherosclerosis

Nana Gletsu and Tapan K. Basu
Department of Food Science and Nutrition, The University of Alberta, Edmonton, Alberta, Canada, T6G 2M8

Abstract

Oxidative modification of low-density lipoproteins (LDL) plays an important role in atherogensis. Oxidized LDL can be formed in vitro by smooth muscle cells, endothelial cells and monocyte-derived cells, all of which are found in the arterial wall. Human and animal atherosclerotic leasions have been found to contain oxidized LDL. It is thought that oxidized LDL particles are ligands for the 'scavanger' receptors on macrophages and can therefore converted to the cholesterol-loaded foam cells characteristic of the early atherosclerotic leasions, the fatty streak. In recent years, the use of nutritional antioxidants, including vitamins E and C, carotenes, and selenium, in the prevention of atheroselerosis has gained hightened interest. Many in vitro experiements and studies in anamals have shown that the antioxidant protect cells from oxidative destruction. Epidemiological studies provide evidence supporting the results from these in vitro and animal studies. The potential anti-atherogenic value of the antioxidant vitamins is further indicated by the inverse relationship between plasma levels of vitamins C and E and carotene, and the risk of coronary heart disease. The scope of this review is to present evidence supporting the use of vitamin antioxidants in the prevention of atherosclerosis, including their mechanisms of action, synergistic effects, and future research merited in this controversial field of study.

Oxygen derived free radicals are the byproducts of aerobic metabolism. A free radical is an atom or molecule with one or more unpaired electron(s). This electron imbalance causes high reactivity, creating other free radicals by chain reactions. These reactive compounds and their products are created through various physiological and biochemical processes such as the mitochondrial respiration, the activation of phagocytes, the biosynthesis of endoperoxides by cyclooxygenase and lipooxygenase, the oxidation of various compounds by enzymes such as xanthine oxidase, and the presence of transition state metals such as Fe^{++} and Cu^{++} (Makheja, 1992; Frie et al., 1989; Cohrane et al., 1988). Oxygen derived free radicals and other pro-oxidants play a positive role in protecting the body against pathogens. But free radicals also inflict damage upon cells by promoting the oxidation of proteins and lipids. In addition to the reactive agents derived from biological processes, humans are also exposed to enviromental sources of pro-oxidants such as cigarette smoke and ultraviolet radiation (Schefller et al., 1992; Frie et al., 1989). There are, however, many naturally occuring substances, which function to protect against the potentially harmful effects of pro-oxidants. These substances, termed antioxidants, can be defined simply as chemical compounds or substances that inhibit oxidation. Antioxidant compounds must be present in biological systems in sufficient concentrations to prevent an accumulation of pro-oxidant molecules, a state known as oxidative stress. In recent years the oxidative stress is increasingly being implicated in the pathogenesis of many degenerative diseases, including coronary heart disease (CHD), cancer, and cataracts (Cerutti, 1985; Gey et al., 1987).

A variety of proteins and enzymes synthesized in the body may function as antioxidants. Such substances include catalase, selenium-dependant glutathione peroxidase, and the transition metal-

Address correspondence to: T.K. Basu, Department of Food Science & Nutrition, 308 Home Economics Building, The University of Alberta, Edmonton, Alberta, Canada, T6G 2M8

binding proteins, transferrin and ceruloplasmin (Krinsky, 1992). Being derived from protein, the levels of these antioxidants are determined by their rate of synthesis, and therefore cannot be manipulated easily. In addition to these endogenous antioxidants, one can obtain oxygen scavengers from dietary sources. The common dietary antioxidants are vitamins C, A, and E, beta-carotene, and selenium. Since these dietary antioxidants are exogenous in nature, their levels can be manipulated by supplementations and dietary modifications. There also appear to be a variety of synthetic compounds such as probucol and butylated hydroxytoluene (BHT), with an antioxidant activity. The use of these compounds, however, is limited due to their possible unwanted side-effects (Finckh et al., 1991; Kita et al., 1987; Ferns et al., 1992; Bjorkhem et al., 1991).

The potential of dietary antioxidants to reduce the risk of coronary vascular disease (CVD), is gaining a great deal of interest in the medical and scientific communities. In this review article the evidence supporting the use of naturally occuring antioxidants with particular references to vitamin C, vitamin E, and the carotenoids in the prevention of atherosclerosis will be discussed.

Oxidative Stress and Atherogenesis

The development of atherosclerotic lesions in the coronary arteries of the heart, if left untreated may lead to CHD, the leading cause of death in affluent communities (Makheja, 1992; Gerster, 1991; Simon, 1992). Atherogenesis is proving to be a very complex process to resolve. One theory is that this process is initiated by oxidative injury to the endothelial lining of the arteries. It is postulated that oxidative stress leading to peroxidation of endothelial membrane lipids, may be cytotoxic to endothelial cells, thus compromising the integrity of the arterial wall (Esterbauer et al., 1991). As part of the inflammatory response, monocytes migrate to the site of injury and begin to infiltrate the area beneath the endothelial lining. Due to some unknown stimulus, these monocytes differentiate into macrophages and begin to imbibe impressive amounts of lipid, whereupon they become foam cells (Esterbauer et al., 1991; Yla-Herttuala, 1991; Parthasarathy et al., 1992; Steinberg, 1991). This process leads to the formation of a fatty streak underneath the endothelium (Stringer et al., 1989). Macrophages also secrete a number of cytokines that promote their own growth and differentiation, and cause the migration of smooth muscle cells into the intimal area (Esterbauer et al., 1991; Yla-Herttuala, 1991; Parthasarathy et al., 1992; Steinberg, 1991). In addition, smooth muscle cells are believed to differentiate into a macrophage-like state, where they start to accumulate lipid and become foam cells. Furthermore, they secrete a number of cell matrix components, such as proteoglycans, which contribute to the hardening of the lesion. At this stage, the lesion becomes a plaque, composed of cells, cell debris and lipid, laying underneath the endothelial lining. A flow diagram of possible oxidation reactions contributing to plaque development is given in Figure 1. Continuous growth of the plaque leads to necrosis of the tissue, which may eventually block the circulation of blood thus causing a heart attack (Esterbauer et al., 1991; Yla-Herttuala, 1991; Parthasarathy et al., 1992; Steinberg, 1991).

Oxidized LDL

It is being realized that the aetiology of lesion formation is multifactorial and complex. Epidemiological evidence suggests high concentrations of cholesterol in the plasma, especially in the form of low density lipoprotein (LDL), is a high predictor of atherosclerotic risk (Esterbauer et al., 1991). Why is LDL atherogenic? An emerging theory (Esterbauer et al., 1991; Yla-Herttuala, 1991; Parthasarathy et al., 1992; Steinberg, 1991; Wiklund et al., 1991) is that under certain conditions, LDL is susceptible to modification particularly by oxidation, and it is this oxidatively modified LDL that triggers foam cell formation (Figure 1). Oxidation of LDL has been shown to occur in vitro upon treatment with metal ions such as Cu^{++} and Fe^{++}, with cigarette smoke, and even with arterial cells, including endothelial, macrophage and smooth muscle cells. In fact,

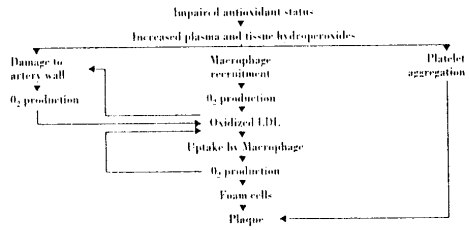

Figure 1: Oxidation Reactions Contributing to Plaque Development.

cigarette smoking, and high serum concentrations of Cu^{++} and Fe^{++}, are believed to correlate positively with incidence of atherosclerosis (Schefller et al., 1992; Oen et al., 1992; Kuzuya et al., 1992).

In addition to endothelial cells other arterial cells are also believed to be affected by oxidative processes, and the changes induced in these cells, may contribute to the progression of atherosclerosis. Oxidative stress has been shown to stimulate chemotactic responses in macrophages and promote smooth muscle cell proliferation and differentiation (Yla-Herttuala, 1991; Parthasarathy et al., 1992; Steinberg, 1991; Chatterjee, 1992; Janero, 1991).

The likely candidate to be responsible for the oxidative modification of LDL and arterial cells, is the oxygen derived free radical (Carpenter et al., 1991; Kukreja et al., 1992; Gunguly et al., 1992). They are believed to interact with the lipid component of LDL particle and cause this lipid to become peroxidatively modified (Oen et al., 1992). Polyunsaturated fatty acids contained within the LDL particle are especially susceptible to be attacked by free-radical species (Yla-Herttuala, 1991; Oen et al., 1992). These reactive compounds are capable of abstracting one of the double allylic hydrogen atoms on the carbon atom between the double bonds of the fatty acid. The reaction then proceeds as depicted below:

I. $LH + R^{\cdot} \rightarrow L^{\cdot}$
II. $L^{\cdot} + O_2 \rightarrow LOO^{\cdot}$
III. $LOO^{\cdot} + LH \rightarrow LOO^{\cdot} + L^{\cdot}$
IV. $LOOH + Fe^{3+} \rightarrow LOO^{\cdot} + H^{+} + Fe^{2+}$
V. $LOOH + 2GSH \rightarrow LOH + GSSG + H_2O$

The product of a reaction between a free radical ($R^{\cdot}$) and a polyunsaturated fatty acid, LH (Equation I), is the pentadienyl radical ($L^{\cdot}$), which can immediately react with oxygen to form the peroxylradical, $LOO^{\cdot}$ (Equation II). This process may be propagated if the peroxylradical reacts with another polyunsaturated fatty acid to form another lipid free-radical and a lipid peroxide, LOOH (Equation III). Lipid peroxides may re-initiate this chain reaction if converted to a free radical (Equation IV). Instead, they may be converted to an alcohol (LOH) by enzymes such as selenium-dependant glutathione peroxidase (GSH), and thus become inactivated (Equation V) (Krinsky, 1992).

The pathological consequences of lipid free-radical and peroxides production have been well documented, and they have recently been implicated for their role in atherogenesis. It has been suggested that the concentrations of lipid peroxides in the plasma correlate well with the severity of atherosclerotic lesions (Stringer et al., 1989; Oen et al., 1992). It is believed that these reactive substances eventually react with apolipoprotein B (apo B), the sole protein component of LDL and promote its oxidation.

Oxidatively modified froms of LDL have been produced in vitro by treating the native lipoprotein with endothelial cells or copper (Steinberg 1989). The physico-chemical properties of this form of LDL (ox-LDL) have been shown to be different from that of native LDL (Esterbäuer et al., 1991). Thus, it has a greater density and increased negative charges compared to native LDL (Esterbauer et al. 1991). The oxidation of lipids in LDL is characterized by the conversion of poly-unsaturated fatty acids into lipid hydroxyperoxides with conjugated double bonds, called conjugated dienes. Further oxidation of LDL causes the conjugation of lysine residues of apo-B with lipid peroxide products such as malondialdehyde, and ultimately the fragmentation of the protein (Esterbauer et al., 1991; Yla-Herttuala, 1991).

What is the physiological significance of this in vitro modified LDL? It may be that LDL is similarily modified *in vivo* under oxidative conditions, and this modified LDL is atherogenic. To test this hypothesis, Yla-Hertualla (1990) extracted LDL from the atherosclerotic lesions of human subjects. This LDL showed physical, chemical and immunological characteristics of *in vitro* oxidized LDL. The finding of this peroxidatively modified lipoprotein predominantly in the atherosclerotic lesions, but not in normal human intima, suggests its atherogenic nature. Additional evidence for the occurrence of LDL oxidation *in vivo*, and its accumulation in atherosclerotic lesions was provided by independant investigators, who identified autoantibodies to malondialdehyde-modified LDL in human serum and in the atherosclerotic lesions of humans and of rabbits (Krinsky, 1992; Parums et al., 1990; Boyd et al., 1989). Furthermore, a recent epidemiological study reported that the titre of autoantibodies recovered from the serum of a group of Finnish men was able to effectively predict the progression of atherosclerosis in these men (Salonen et al., 1992).

Since the aetiology of atherosclerosis seems to involve lipid peroxidation it is conceivable that processes that limit lipid-peroxidative processes, may retard or prevent the progression of atherosclerosis. In general, antioxidants function by impairing oxidative chain reactions at one or more of the outlined steps involved in lipid peroxidation, converting reactive radical species to relatively inert compounds. The major biological antioxidants are the lipid-soluble group such as vitamin E and the carotenoids, and plasma-soluble vitamin, ascorbic acid.

Vitamin E (tocopherols)

The vitamin E family of antioxidants are lipid soluble substances chiefly found in the plasma in circulating lipoproteins, and in the lipid of cell membranes. The most commonly found members of the vitamin E family in humans are the group of tocopherols, alpha-tocopherol being the most abundant and the most active isomer in this group (Esterbauer et al., 1991). The other group, the tocotrienols, are more highly unsaturated, and possibly more potent antioxidants, however, they are present in much smaller quantities (Olson, 1992). Once absorbed from the gut, vitamin E is transported to target tissues and cells via the lipoproteins. Amoung them, it is estimated that LDL carries about 50 % of circulating vitamin E (Esterbauer et al., 1991). Normally, an average of six molecules of alpha-tocopherol, are present in each LDL particle (Esterbauer et al., 1991). Other lipid soluble antioxidants, including gamma-tocopherol, beta-carotene, and alpha-carotene are found in LDL in much lower quantities, one-twelfth, one-twentieth, and one-fiftieth, respectively of the alpha-tocopherol concentration (Esterbauer et al., 1991). The proximity of vitamin E to lipids in LDL explains its unique ability to directly prevent lipid peroxidation within the LDL particle).

Vitamin E functions by donating hydrogen to fatty-peroxyl radicals, thereby halting lipid peroxidation. This antioxidant can potentially react with two peroxyl radicals, as shown below:

VI). α-tocopherol + LOO· -> α-tocopherol· + LOOH

VII). α-tocopherol· + LOO· -> LOO-α-tocopherol

The alpha-tocopherol radical (α-tocopherol·) produced in Equation VI, assumes a resonance-stabilized conformation, enabling the molecule to react with another peroxyl radical to form a stable adduct, LOO-α-tocopherol (Equation VII) (Krinsky, 1992).

Vitamin E has been touted as the most potent biological lipid antioxidant (Steinberg, 1991; Janero, 1991). An abundance of evidence exists to support this claim. Epidemiological studies have shown that vitamin E is the strongest contributor to the inverse relationship between serum antioxidant concentrations and Ischemic Heart Disease (IHD) (Esterbauer et al., 1991; Steinberg, 1991; Gey, 1990). A recent eight-year follow-up study involving 87,245 female nurses has also found that women with highest intake of vitamin E had a relative risk of CHD, 0.66 of that of the group which consumed the least amount of the vitamin, after adjustment for age and smoking (Stampfer et al., 1993). Experimentally, it has been shown, that dietary vitamin E can ameliorate the development of spontaneous atherosclerosis in nutritional models of CVD (Verlangieri and Bush 1992). Atherosclerotic lesions in the carotic arteries were induced in a primate model over a period of time by feeding the animals a high cholesterol diet. Alpha-tocopherol supplementation was shown to decrease the severity of atherosclerosis and promote the regression of diet induced atheroslcerotic lesions. Similar results were described in a rabbit model (Rozewicka et al., 1991). Vitamin E has also been shown to inhibit oxidative LDL modification. In a rabbit model, experimental atherosclerosis was induced *in vivo* by cholesterol feeding, and *in situ* by mechanical injury to segments of aorta tissue caused by a perfusion system. The perfused aortas were then incubated in the presence or absence of alpha-tocopherol, and the cellular uptake of LDL was determined. Alpha-tocopherol decreased the uptake of LDL into plaques and into foam cells, leading the investigators to believe that its antioxidant activity was responsible. It must be noted however, that the authors were unable to provide direct evidence that vitamin E inhibited LDL oxidation. Numerous studies have reported that chronic vitamin E deficiency in animals elicits atherosclerotic like lesions which can be reduced by vitamin E supplementation (Nelson, 1980; Liu et al., 1984).

In a primate model, inadequate dietary supplementation of vitamin E and abnormally low plasma alpha-tocopherol content ensued in heart failure and death (Liu et al., 1984). Examination of the cardiac tissue revealed fatty streaks in the myocardium of all animals examined. Increased vitamin E supplementation of three deficient animals restored serum values to normal levels and prevented subsequent death from cardiac disease. Similar studies have been described in rodents and piglets (Nelson, 1980). In humans, levels of vitamin E in the plasma have been shown to correlate inversely with the levels of serum lipid peroxides. Indeed, dietary vitamin E administration was found to decrease the susceptibility of LDL isolated from hypercholesterolemic men to oxidative modification (Fruchart et al., 1989). Elderly people, who were given vitamin E supplementation of 200 mg/day, were observed to have a 26 % decrease in serum peroxide levels after one year of supplementation (Wartanowicz et al., 1984). Supplementation of the human diet with vitamin E (600 mg/day), had a protective effect on the *in vivo* oxidation of LDL caused by acute smoking (Harats et al., 1989). Harats study demonstated that thiobarbituric acid reactive lipid peroxidation by-products (TBARS) in plasma and in LDL after ninety min of acute smoking, were significantly reduced in subjects receiving alpha-tocopherol supplementation (Scheffler et al., 1992; Schuh et al., 1978). Treatment of LDL with vitamin E, either *in vivo*, by supplementing the diet with the vitamin, or in vitro, by incubation of the lipoprotein with vitamin E, has been demonstrated to reduce the rate of TBARS formation in LDL samples incubated with Cu^{++} (Esterbauer et al., 1991). There is also evidence, that species with relatively low vitamin E levels, such as that seen when comparing porcine to human LDL, have increased susceptibility to LDL modification (Janero, 1991). Taken together, this evidence suggests that alpha-tocopherol contributes the most to the antioxidant protection of LDL in biological systems. As the LDL particle is subjected to oxidative stress, its endogenous antioxidants, mainly alpha-tocopherol, are consumed first (Esterbauer et al., 1991). The onset of lipid peroxidation, demonstrated by TBARS formation, only occurs after LDL is depleted of these antioxidants (Esterbauer et al., 1991).

Although vitamin E plays a substantial role in preventing lipid peroxidation, it relies upon the availability of other antioxidants to be able to function normally. The alpha-tocopherol radical can be regenerated to active alpha-tocopherol by reacting with ascorbate (Esterbauer et al., 1991; Sies et al., 1992). The reaction of ascorbate (AH^-) and the alpha-tocopherol alkoxy radical ($\alpha\text{-TO}^{\cdot -}$), to yield active alpha-tocopherol ($\alpha\text{-TO}^{\cdot}$) and semi-dehydroascorbate radical ($A^{\cdot -}$) is depicted in equation VIII (Frei et al., 1989):

VIII. $AH^- + \alpha\text{-TO}^{\cdot} \rightarrow \alpha\text{-TOH} + A^{\cdot -}$

In this way, the plasma-soluble antioxidant sacrifices its activity to ensure that adequate levels of alpha-tocopherol are maintained in the membrane fraction. Therefore ascorbate plays a major role in protecting against lipid peroxidation. It has been shown experimentally, that the presence of ascorbate in LDL samples subjected to oxidative stress, was vital in preventing lipid peroxidation, even when the LDL contained vitamin E. The presence of ascorbate in the incubation mixture of LDL and Cu^{++}, was shown to inhibit TBARS formation when compared to LDL samples incubated without ascorbate, and to preserve 69 % of the original LDL levels of alpha-tocopherol (37).

Selenium is another antioxidant which is involved in vitamin E function. This micronutrient is necessary component of the enzyme glutathione peroxidase (GSH). Like vitamin E, selenium-dependant glutathione peroxidase functions to inactivate lipid peroxides. The enzyme catalyzes the conversion of lipid peroxides (LOOH) to relatively inert alcohol forms (LOH) as shown in equation IX (Krinsky, 1992).

IX. LOOH + 2GSH -> LOH + GSSG + H_2O

Adequate concentration of selenium must be available for glutathione to function properly. Evidence of the antiatherogenic property of selenuim is abundant (Rozewicka et al., 1991; Suadicani et al., 1992; Salonen et al., 1991). In two epidemiological studies, high incidence of atherosclerosis and CHD were observed in populations with low serum selenium concentrations compared with those with adequate selenium (Salonen et al., 1991; Suadicani et al., 1992). In one of these studies, hyperlipidemic subjects with low selenium concentrations had an even more exagerated risk of CHD (Salonen et al., 1991). Possible reasons for the effect seen in these subjects, may be the increased amounts of LDL in their serum which required increased protection from oxidative stress.

Carotenoids

Like vitamin E, the carotenoids, including beta-carotene, gamma-carotene, and lycopene, are lipid soluble, and are therefore carried within lipoprotein particles. Normally one mole of LDL contains only about 0.29 mol of beta-carotene, 0.12 mol of alpha-carotene, and 0.16 mol of lycopene; small amounts relative to that of alpha-tocopherol (Gerster, 1991; Esterbauer et al., 1991). The idea that like vitamin E, beta-carotene may prevent LDL oxidation, is fairly new. In fact beta-carotene is known more for its pro-vitamin A activity (Gerster, 1991). However, because carotenoids feature a conjugated double-bond system, they are highly efficient quenchers of singlet oxygen ($^1O_2$) (Canfield et al., 1992). At the low oxygen pressures (15 to 160 torr O_2), normally present in the physiological situation, carotenoids are also excellent substrate for free radical attack (Krinsky, 1992; Sies et al., 1992). Beta-carotene has the added advantage of being able to trap more lipid free-radicals when compared to alpha-tocopherol, the latter being able to trap a maximum of two (Krinsky, 1992). This is because of beta-carotene's ability to form multiple resonance stabilized molecules as subsequent binding of free radicals occurs. The reaction of beta-carotene (CAR) with lipid peroxyl radicals (LOO·), to form multiple carbon-centered radicals (LOO-CAR·, LOO-CAR-OOL, $(LOO)_2$-CAR-OOL·, and $(LOO)_2$-CAR-$(OOL)_2$) is shown below (Krinsky, 1992):

X. CAR + LOO· -> LOO-CAR·

XI. LOO-CAR· + LOO -> LOO-CAR-OOL

XII. LOO-CAR-OOL + LOO· -> $(LOO)_2$-CAR-OOL·

XIII. $(LOO)_2$-CAR-OOL· + LOO· -> $(LOO)_2$-CAR-$(OOL)_2$

Another unique ability of the carotenoids is worth noting. As carotenoids trap singlet oxygen, they release energy in the form of heat, thus a regeneration system, such as that required for alpha-tocopherol is not needed (Sies et al., 1992). Even though these properties suggest that beta-carotene may have a more potent antioxidant activity than alpha-tocopherol, at the present time, it is more conceivable that it makes less of a contribution to the protection of LDL *in vivo*. As previously mentioned, the concentration of beta-carotene in LDL is one-twentieth that of alpha-tocopherol (Esterbauer et al., 1991). In addition, the scant evidence that has been gathered concerning beta-carotene's antioxidant potential, has been contradictory. Some studies have failed to show that the presence of beta-carotene in a LDL preparation was able to inhibit the oxidation induced by free radicals (Morel et al., 1983; van Hindsberg et al., 1986). In a more recent study by Jialal et al., (1991), when hexane was employed to solubilize beta-carotene instead of ethanol, it was shown that 2 uM of beta-carotene was more potent than alpha-tocopherol (40 uM) in inhibiting the oxidative modification of LDL. Possible reasons for the discrepancy of the results in these studies may lie in the difficulty of getting beta-carotene into solution, and its potential to act as a pro-oxidant at high concentrations and at high oxygen pressure (760 torr) (Steinberg, 1992; Sies et al., 1992). Few studies have been reported using beta-carotene in animal models and in humans. Recently, a large case-controlled study involving physicians, reported preliminary findings of reduced cardiac myopathy in a beta-carotene supplemented group (Hennekens and Eberlein, 1985). In this study, subjects with a history of cardiac myopathy were randomized into a beta-carotene supplemented group (50 mg / every two days), and a placebo group. A statistically significant reduction in cardiovascular events of almost 50 % was observed in the beta-carotene group when compared with the placebo group. At the dosage used in this study, no unwanted side effects were noted. These findings, along with evidence that beta-carotene may reduce the incidence of certain cancers suspected to have free radical involvement, lend support to the concept that beta-carotene may be involved in the protection against atherosclerosis, possibly due to its antioxidant action (Temple and Basu, 1988). Further investigation is warranted to define beta-carotene's role in atherosclerosis prevention and therapy.

Vitamin C (ascorbate)

As a water-soluble compound, ascorbic acid may very well be the front-line of defence against free-radicals created by metabolism. Ascorbate has been found to react with hydrogen peroxide (H_2O_2), the hydroxyl radical ($OH\cdot$), peroxyl radicals ($ROO\cdot$) and singlet oxygen ($O_2\cdot^-$), to form the semidehydroascorbate radical (A^-) and dehydroascorbate (A) as depicted below (Sies et al., 1992; Stadtman, 1991):

XIV. $AH^- + OH \rightarrow H_2O + A\cdot^-$
XV. $AH^- + O_2^- + H^+ \rightarrow H_2O_2 + A\cdot^-$
XVI. $AH^- + ROO\cdot \rightarrow RH + A\cdot^-$
XVII. $AH^- + H_2O_2 + H^+ \rightarrow 2H_2O + A$

This water soluble antioxidant acts in the plasma to scavenge free radicals, dissipating these reactive species before they can react with biological membranes and lipoproteins. This was demonstrated by a study in which human blood plasma exposed to a water soluble radical initiator, or to the oxidants generated by polymorphonuclear leukocytes, did not show any signs of lipid peroxidation as long as ascorbate was present (Frei et al., 1989). In addition, plasma devoid of ascorbate but no other endogenous antioxidants, such as alpha-tocopherol, was found to be extremely susceptible to lipid peroxidative damage (Frei et al., 1989). Animal studies have reported that marginal ascorbate deficiency induced by diet leads to myocardial injury as evidenced by lipid peroxidation, this damage was prevented by ascorbic acid supplementation (Chakrabarty et al., 1992). Such observations have also been made in human studies. Elderly subjects receiving 400 mg of vitamin C daily, experienced a 13% decrease in serum peroxide levels after one year (Wartanowicz et al., 1984). Similarly, vitamin C supplementation of 1.5 g/day for 4 weeks, was shown to protect against the rise in plasma and LDL TBARS associated with acute cigarette smoking (Harats et al., 1989). Ascorbate also has the ability to regenerate the activity of lipid soluble antioxidants, such as gamma-tocopherol and beta-carotene, by interacting with biological membranes at the aqueous-lipid interphase (Steinberg, 1989; Stadtman, 1991; Jialal and Grundy, 1991). This feature of vitamin C has earned it the title of a sacrificial antioxidant. The sparing effect of ascorbate on lipid soluble antioxidants has been demonstrated *in vitro*. In one investigation, treatment of LDL with 10 uM ascorbate spared lipid soluble antioxidant activity for about 90 minutes, only after the consumption of ascorbate did the first lipid hydroxyperoxides become detectible (Gey, 1990). Consistent with this finding, studies have reported alpha-tocopherol to be ineffective in preventing lipid peroxidation in cardiac tissue that contained relatively low levels of vitamin

C (Simon, 1992; Sies et al., 1992; Jialal and Grundy, 1991). Taken together, this evidence suggests that ascorbate may have a dual antioxidant function in biological systems; to inactivate damaging radicals in the plasma, and to preserve the activity of lipophilic antioxidants.

Epidemiological studies report an inverse relationship between vitamin C intake and CHD mortality (Simon, 1992). However the mechanisms responsible for this effect appear to be complex. In addition to preventing lipid peroxidation, ascorbate has been shown to affect other potential risk factors associated with the disease. There is some evidence that vitamin C lowers plasma total cholesterol by promoting its conversion to bile acids (Ginter, 1975). In addition, the atherosclerotic lesions seen in animals and humans with chronic vitamin C deficiency, may result from increased vascular fragility secondary to a deficiency in collagen synthesis (Murad et al., 1981). Vitamin C supplementation has also been shown to lower blood pressure, possibly by influencing prostaglandin synthesis (Jacques, 1992). Thus vitamin C may have additional antiatherosclerotic effects that may be unrelated to its antioxidant activity.

Conclusions

Lipid-soluble antioxidants such as tocopherols and carotenoids, appear to prevent lipid peroxidation in biological membranes. The tocopherols, may play the most important role in this respect, however their activity largely depends upon the presence of other nutrients, such as ascorbate and selenuim.

A large body of evidence exists to suggest that antioxidants, by inhibiting LDL oxidation, may protect against the development of atherosclerosis. Furthermore, it appears that in the biological system, the effectiveness of each antioxidant not only depends on its reactivity and concentration, but on the availability of regenerating systems. A recent study examined the oxidizability of LDL in human volunteers supplemented with beta-carotene (18 mg), ascorbic acid (900 mg) and alpha-tocopherol (200 mg) daily for six months (Abbey et al., 1993). This study has demonstrated that the vitamin antioxidants mixture protects LDL from oxidation in vitro by increasing the lag time before onset of oxidation. However, there appears to be a wide variability among lipoproteins from individuals in susceptibility to oxidation as well as in incorporation of antioxidant vitamins, such as vitamin E into lipoproteins (Jessup et al., 1990). A variety of factors such as lipid composition and turnover rate of lipoproteins may be responsible for such a variation.

Although several-case controlled studies lend support to the possible role of vitamin E, ascorbate and beta-carotene in the reduction of IHD risk, no clinical studies have been done to determine the effect of combined supplementation of these antioxidants. Recently a National Heart Lung and Blood Institute-sponsored conference (Steinberg, 1992), concluded that a randomized trial, using a 2x2x2 factorial approach to determine the individual as well as the combined effects of vitamin E, ascorbate and beta carotene was warranted. Because these micronutrients, may be used at therapeutic dosages, with little or no toxic side effects, such a trial is feasible. In the meantime, more research is needed on the mechanisms involved in the oxidative modification of LDL and the ways in which various antioxidants influence it.

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