ANTIOXIDANTS IN PREVENTION AND TREATMENT OF CARDIOVASCULAR DISEASE

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INTRODUCTION

Cardiovascular disease remains the leading cause of death worldwide, despite major therapeutic advances (in particular, the development of anti-platelet agents, thrombolytic drugs and angiotensin converting enzyme inhibitors). Population trends toward increased longevity and greater consequent exposure to atherosclerotic risk factors mean that prevention of cardiovascular disease will assume even greater importance in the future. Substantial progress has been made over recent years in identifying mechanisms involved in the development of atherosclerosis, including oxidative stress, a characteristic finding in patients with major cardiovascular risk factors such as diabetes mellitus, hypertension, hypercholesterolaemia and smoking. Observational findings from epidemiological studies suggest that antioxidant supplementation could protect against the potentially deleterious effects of oxidative stress on the cardiovascular system, thus preventing the development of atherosclerosis and cardiovascular disease. Further evidence is accumulating from randomised, controlled, clinical trials that antioxidant supplementation, in a variety of forms, may have a favourable influence on preventing the development of cardiovascular disease. This article develops the rationale for antioxidant therapy in the prevention of cardiovascular disease, and discusses the implications of data emerging from clinical trials.

OXIDANTS IN THE CARDIOVASCULAR SYSTEM

An oxidant is any substance capable of removing electrons from adjacent molecules, and in humans the most potent oxidants are 'free radicals'. Free radicals are capable of existence with one or more unpaired outer shell electrons, and are highly unstable and reactive. Oxygen-derived free radicals are of greatest biological significance, often arising as a consequence of inefficient oxidative metabolism, and include superoxide ($O_2\cdot$-), hydrogen peroxide (H_2O_2) and the potent hydroxyl free radical ($OH\cdot$-). Molecules subjected to free radical attack will be left with an unpaired outer shell electron, having themselves become free radicals, and may be capable of sustaining a redox chain reaction depending on their own reactivity. In this way oxidants can cause severe disruption of cellular function; in the cardiovascular system the most harmful effects are mediated through lipoprotein oxidation, and oxidative degradation of nitric oxide (NO), a molecule serving an important protective physiological role.

Lipid peroxidation
Lipoproteins are particularly vulnerable to attack by free radicals, where polyunsaturated fatty acids are oxidised at unsaturated carbon double bond sites. These target molecules react rapidly with oxygen to form peroxyl radicals, which in turn further react with fatty acids and generate lipid peroxides. Lipid peroxides are unstable

radicals capable of propagating redox chain reactions; when they decompose they form toxic secondary products that can cause direct tissue damage. Measurement of secondary product concentrations, for example serum malon-dialdehyde, indicates the extent of systemic lipid peroxidation and thus provides a marker of oxidant activity within the cardiovascular system.[1]

Degradation of NO
Nitric oxide is an important regulatory molecule synthesised by the action of nitric oxide synthase (NOS) and released by healthy endothelium. N^G-monomethyl-L-arginine (L-NMMA) competitively inhibits NOS, and has provided a pharmacological tool for evaluation of the physiological actions of NO, which include vascular smooth muscle relaxation, inhibition of platelet aggregation and inhibition of myointimal hyperplasia.[2] Reduction of NO bioavailability by L-NMMA administration accelerates atherosclerosis development in animal models, while enhancing NO bioavailability by administration of its precursor, L-arginine, delays progression.[3] Therefore, loss of physiological NO bioavailability appears to be an important early step in the development of atherosclerosis, and this finding has been consistently observed in the forearm vascular bed of patients with diabetes mellitus,[4] hypercholesterolaemia[5] or hypertension,[6] all of whom have a greatly increased cardiovascular risk, as do smokers.[7] Nitric oxide is normally subjected to rapid degradation, and is particularly susceptible to inactivation by oxidation.[8] The characteristically increased oxidant exposure in these conditions appears to be responsible for the observed loss of NO bioavailability, through accelerated oxidative degradation.

OXIDATIVE-MODIFICATION THEORY OF ATHERO-SCLEROSIS

Development of atherosclerosis is a multifactorial process that depends on both environmental factors and constitutional factors, which are in part inherited. Formation of precursors to atherosclerotic plaques occurs early in life, and one of the first identifiable steps in plaque development is endothelial cell injury, which may be a consequence of direct free radical damage, or the toxic action of secondary products of lipid peroxidation. Loss of endothelial integrity predisposes to platelet adherence, release of chemotactic agents and enhanced sub-endothelial accumulation of cholesterol and cholesterol esters (Figure 1). Under the influence of local oxidants, sub-endothelial native LDL is converted to oxidised LDL, which is a potent chemo-attractant stimulus for macrophage migration and smooth muscle proliferation, and is toxic to the endothelium, thus promoting further LDL deposition and atheromators plaque propagation. Sub-endothelial macrophages ingest native LDL cholesterol via a receptor-mediated pathway, while oxidised LDL is ingested by a separate 'scavenger' pathway
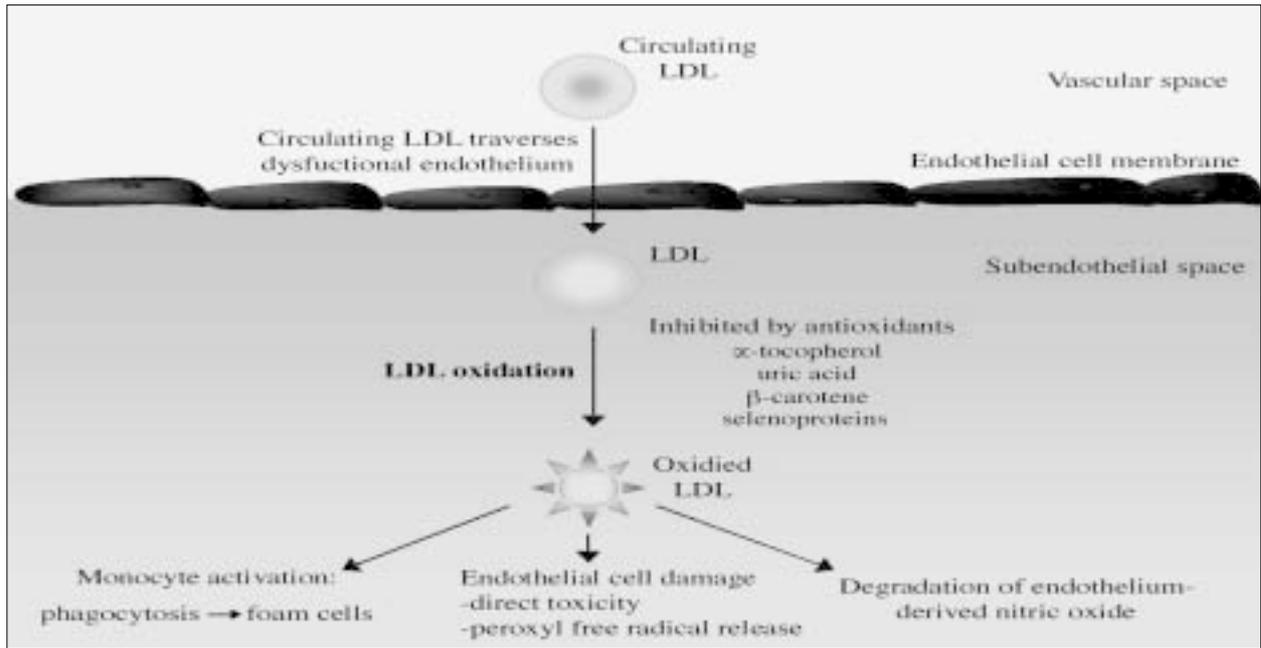
FIGURE 1

Schematic representation of how oxidants and antioxidants are linked to disruption of endothelial integrity, an important early step in the development of atherosclerosis.

that results in formation of cholesterol-saturated macrophages, or foam cells, a characteristic pathological finding in atherosclerotic plaques.

Therefore sub-endothelial LDL oxidation is a central step in the oxidative-modification model of atherosclerosis and has prompted considerable interest in factors determining local oxidant activity, and factors that may influence susceptibility of LDL to oxidation.[9]

ANTIOXIDANT DEFENCES

A number of important physiological antioxidant systems serve to oppose the effects of free radicals and oxidants on target substrates. Those capable of sequestering transition metal ions, which in a free form catalyse formation of hydroxyl radicals, can be considered as preventative antioxidants; they include the copper-binding protein caeruloplasmin, the iron-binding protein transferrin, and albumin. Enzymatic antioxidants catalyse the breakdown of oxidants within cells, and include superoxide dismutase, catalase and glutathione peroxidase. Non-enzymatic, or 'scavenging', antioxidants are consumed during quenching of free radicals and oxidants, thus preventing organ damage. These sacrificial antioxidants can conveniently be considered as water-soluble or lipid-soluble agents, thereby partly reflecting the respective degree to which each is active in aqueous or lipid-rich compartments (Table 1).

Aqueous antioxidants

The most important physiological aqueous antioxidants are vitamin C (ascorbic acid) and uric acid, while bilirubin and thiol-containing molecules make a comparatively small contribution. Humans are unable to synthesise vitamin C and therefore depend solely on its dietary intake; fresh fruit and vegetables provide the richest source. Vitamin C is the most potent electron donor among scavenging free radicals, and is widely distributed intracellularly and extracellularly, and can be regenerated from its oxidised form, dehydroascorbate.[10] Uric acid is also widely

distributed in extracellular fluid and found in significantly higher concentrations than vitamin C. Uric acid is formed by purine metabolism and, since humans do not express the enzyme urate oxidase, we are exposed to significantly higher concentrations than virtually all other species. As a result of high concentrations, uric acid provides the most abundant source of scavenging activity in humans, for example contributing up to two-thirds of all serum free radical scavenging capacity.[11] The physiological role of uric acid remains poorly understood, although higher serum concentrations may be an adaptive response to increased oxidant exposure that results from longevity and environmental changes.[12]

Lipophilic antioxidants

Vitamin E refers to tocotrienols, of which α-tocopherol is the most potent. It is obtained from dietary sources that

TABLE 1

Physiologically active oxidants and antioxidants that may play a significant role in the cardiovascular system.

Oxidants	Antioxidants
Primary radicals	*Antioxidant enzymes*
Superoxide	Superoxide dismutase
Peroxide	Catalase
Hydroxy radical	Gluatathione peroxidase
Secondary radicals	*Scavenging antioxidants*
Lipid peroxide and hydroperoxide	*Aqueous:* Uric acid, Ascorbic acid (vitamin C), Bilirubin
	Lipophillic: α-tocopherol, β-carotene (vitamin E), Ubiquinol-10

include cereals, milk, liver and vegetable oils, and provides the most important physiological antioxidant defence within lipoproteins and cell membranes. Vitamin E defences can rapidly become depleted but transfer of electrons from vitamin C, at the interface between lipid and aqueous compartments, allows its regeneration to an active form. Carotenoids, including β-carotene, circulate in lipoproteins and provide a comparatively small contribution to antioxidant activity in man. Ubiquinol-10 (reduced co-enzyme Q_{10}) is present in small quantities and may also have an important role in the regeneration of active vitamin E.

Inadequate antioxidant defences
Oxidative stress is a term used to describe an unfavourable imbalance between potentially harmful oxidants and protective antioxidants. This is a characteristic feature of conditions associated with increased cardiovascular risk and thought to result primarily from increased vascular oxidant activity. Often associated is a reduction in circulating antioxidant defences although this may result from consumption in the presence of increased oxidant activity. For example, low serum concentrations of ascorbic acid are found in smokers,[13] and insulin-dependent diabetic patients have low serum uric acid concentrations.[11] The presence of multiple major cardiovascular risk factors is associated with a synergistic increase in oxidative stress[14] and consequent tissue damage, indicated by increased circulating lipid peroxidation products.[15]

EVIDENCE SUGGESTING A PROTECTIVE ROLE OF SUPPLEMENTARY ANTIOXIDANT TREATMENT
Studies comparing fruit and vegetable consumption across different nations have identified an inverse relationship between total intake and risk of cardiovascular mortality. Consistent findings have been demonstrated within populations, for example comparison of nine UK regions has shown a strong inverse correlation between fresh fruit and vegetable intake, and the incidence of coronary heart disease.[16] These studies have, however, been limited by several confounding factors, since fresh fruit and vegetables may contain other protective elements unrelated to antioxidant content, and preference for a diet with a low saturated fat content may confer important cardiovascular protection irrespective of the consumption of fresh fruit and vegetables. Dietary antioxidant consumption is also likely to be influenced by economic factors, and be associated with lifestyle characteristics that influence health, for example exercise and smoking patterns. A more direct relationship with antioxidant vitamins was identified by the World Health Organisation project to monitor trends in cardiovascular disease (MONICA). This showed a strong inverse relation between coronary heart disease mortality rates and measured serum vitamin E ($R^2 = 0.63$, $p = 0.002$) and vitamin C concentrations ($R^2 = 0.73$, $p = 0.0004$) across 12 European populations.[17]

Observational data has been obtained from 87,245 healthy female nurses who underwent an assessment of dietary antioxidant intake and were studied over an eight year period.[18] After adjustment for age and smoking status the relative risk reduction for a coronary event in the highest versus lowest quintile of vitamin E consumption was 34% (95% CI 13–50%), and benefits were attributed to vitamin E supplementation (>100 IU per day) rather than dietary intake alone. Vitamin C had no apparent protective effect.

An observational study of 39,910 male health professionals over four years identified a relative coronary heart disease risk reduction of 36% (95% CI 16–53%) in those taking vitamin E supplementation (>100 IU per day), and a trend towards reduced risk in those with highest dietary vitamin E consumption.[19]

Epidemiological observations have highlighted the important association between dietary antioxidant consumption and reduced cardiovascular risk, and observational studies support the view that dietary antioxidant supplementation is associated with reduced coronary heart disease risk in both males and females. These findings have prompted prospective controlled clinical trials to study the possibility that antioxidant supplementation could prevent the development or progression of cardiovascular disease.

PROSPECTIVE TRIALS OF ANTIOXIDANT SUPPLEMENTATION IN APPARENTLY HEALTHY PEOPLE
The Women's Health Study was a multifactorial, double blind, placebo-controlled study of the effects of aspirin, α-tocopherol (600 mg on alternate days), and β-carotene (50 mg on alternate days) on the incidence of cardiovascular disease and cancer in 39,876 healthy women aged 45 years or over.[20] After a mean of 2.1 years of treatment and a further mean 2 years of follow-up, there was no significant difference in the incidence of myocardial infarction (0.25% and 0.21%), stroke (0.22% and 0.31%), cardiovascular death (0.06% and 0.07%) or overall death (0.28% and 0.30%) between placebo and β-carotene treated groups.[20]

The Physicians Health Study (PHS) was a prospective placebo-controlled study of the effects of β-carotene 50 mg alternate days (and/or aspirin in a separate arm) on prevention of cardiovascular disease and cancer. For this study, 22,071 males without previous myocardial infarction or stroke were recruited in 1982 and studied for a mean of 12 years.[21] No significant difference was found between the β-carotene or placebo-treated groups in the incidence of myocardial infarction (4.43% and 4.24%), stroke (3.46% and 3.33%), cardiovascular deaths (2.84% and 3.06%) or overall mortality (8.77% and 8.87%).[21] The PHS-II is a subsequent multifactorial study that is currently underway to study the effects of alternate day β-carotene, alternate day vitamin E, daily vitamin C, or a daily multivitamin in the prevention of cardiovascular disease, cancer, and age-related ocular disease.[22]

PROSPECTIVE TRIALS OF ANTIOXIDANT SUPPLEMENTATION IN HIGH RISK GROUPS
Among patients with established cardiovascular disease entered into the PHS study, β-carotene treatment significantly improved outcome compared to placebo. Follow-up of these 333 patients over 60.2 months showed a relative risk reduction of 51% (95% CI 12–71%) for all coronary heart disease events.[23] This study suggested that antioxidant supplementation may lead to significant benefits in those patients with established cardiovascular disease.

In the Alpha-Tocopherol Beta-Carotene (ATBC) study 1,862 male smokers aged 50 to 69, who had had a previous myocardial infarction, were randomised to receive placebo or α-tocopherol 50 mg daily, or β-carotene 20 mg daily, or both for a median follow-up of 5.3 years. In contrast to the PHS subgroup study, there was no significant difference between placebo and any other group in the incidence of

non-fatal myocardial infarction (12·6%, 8·6%, 8·5% and 11·3% respectively) or fatal coronary heart disease (9·26%, 11·6%, 16·1% and 13·5% respectively).[24]

In the Cambridge Heart Antioxidant Study (CHAOS) 2,002 patients with angiographically-proven coronary artery disease were randomised to receive placebo or α-tocopherol 400–800 IU (268–537 mg) daily and followed-up for a median 510 day period. Alpha-tocopherol led to a significant risk reduction of 47% (95% CI 17–66%) for cardiovascular death and non-fatal myocardial infarction, including a risk reduction of 77% (95% CI 53–89%) in the incidence of non-fatal myocardial infarction.[25] Cardiovascular mortality (2·4% and 2·6%) and overall mortality rates (2·7% and 3·5%) were similar in those treated with placebo or α-tocopherol, although analysis on the basis of treatment compliance suggested a more substantial mortality reduction in the α-tocopherol group.[26] The CHAOS study showed that antioxidant supplementation confers a substantial reduction of future cardiovascular events among patients at high risk of cardiovascular disease, and may have failed to show an overall survival benefit due to the comparatively short follow-up period.

In the Secondary Prevention with Antioxidants of Cardiovascular disease in Endstage renal disease study (SPACE), 196 haemodialysis patients with established cardiovascular disease were randomised to receive placebo or vitamin E supplementation (800 IU per day) for a median of 519 days. The composite primary end-point (myocardial infarction, angina, peripheral vascular disease and stroke) occurred in 33% (33 of 99) of those assigned to placebo and in 16% (15 of 97) of those receiving vitamin E supplementation, representing a relative risk reduction of 54% (95% CI 22–73%).[27] Myocardial infarction occurred in 17·2% (17 of 99) and 5·1% (5 of 97) respectively, a relative risk reduction of 70% (95% CI 22–89%).[27] Results from SPACE and CHAOS were remarkably consistent, and suggest that supplementary vitamin E treatment may be of value in patients with high cardiovascular risk.

Two large studies have reported the effects of long-term α-tocopherol supplementation in patients at high risk of cardiovascular disease. The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico study (GISSI prevenzione) trial randomised 11,324 patients (85% male) with previous myocardial infarction to receive placebo, or α-tocopherol (300 mg daily), or n-3 polyunsaturated fatty acid (1g daily), or both for a mean follow-up of 3·5 years.[28] The Heart Outcomes Prevention Evaluation (HOPE) study randomised 9,541 patients (73% male) with established cardiovascular disease to receive placebo or vitamin E (400 IU daily) for a mean follow-up of 4·5 years.[29] Both of these large prospective studies found that the occurrence of myocardial infarction, stroke, cardiovascular death and overall mortality was similar in α-tocopherol and placebo treated groups, although a trend towards reduced sudden cardiac deaths was observed.[28] Therefore, in contrast to both CHAOS and SPACE, long-term supplementation with vitamin E did not reduce the incidence of myocardial infarction in patients with moderately increased cardiovascular risk.

SUMMARY
Epidemiological data show an inverse correlation between cardiovascular disease risk and dietary antioxidant consumption, suggesting that antioxidant supplementation

may have protective effects within the cardiovascular system. However, it has been difficult to extrapolate these findings into clinical practice due to possible confounding factors, and because dietary patterns are likely to have been established early in life and maintained over many years. Oxidative stress appears to play a key role in the development of atherosclerosis through impairment of endothelial function, oxidation of LDL cholesterol and promotion of vascular inflammation. Despite these findings, clinical studies have failed to demonstrate a consistent benefit from antioxidant supplementation. This may be in part due to the comparatively short treatment periods of some studies; further inconsistency may have been introduced by variation in the form or dosage of antioxidant chosen. For example, it is possible that the observed benefits in the CHAOS study reflect the higher potency of α-tocopherol, compared to naturally occurring vitamin E as used in the HOPE study. Similarly, the benefits observed in the CHAOS and SPACE studies could be accounted for by a higher average administered dosage of vitamin E than used in either the HOPE or GISSI prevenzione studies. In the latter studies, confounding effects of non-compliance and open-label antioxidant treatment cannot be excluded, since serum α-tocopherol concentrations were not measured.[30] Background cardiovascular mortality in the CHAOS and SPACE studies was significantly higher than in the HOPE or GISSI prevenzione studies and, therefore, the chosen patient selection criteria may also influence the response to treatment.

The potential benefits of any single antioxidant may be outweighed by supplementary combined treatment, and large prospective studies of combined antioxidant treatment for primary and secondary cardiovascular disease prevention are underway. The Supplementation en Vitamines et Mineraux Antioxydants (SU.VI.MAX) study is a placebo-controlled study of the effect of combined antioxidant treatment (vitamin C 120 mg, vitamin E 30 mg, β-carotene 6 mg, selenium 100 mg, zinc 20 mg) on the incidence of cardiovascular disease in a healthy population; 12,735 healthy men and women were recruited in 1994 for intended eight year follow-up.[31] In addition, the joint BHF/MRC Heart Protection Study is a prospective multifactorial trial of daily multivitamin (600 mg vitamin E, 250 mg vitamin C and 20 mg β-carotene) and/or simvastatin treatment on the incidence of coronary heart disease and stroke in a population with established cardiovascular disease.[32] For this study, 20,536 patients (75% male) were recruited between 1994 and 1997 for intended five year follow-up, all having at least one major cardiovascular risk factor; myocardial infarction (41%), other coronary heart disease (16%), peripheral vascular disease (29%), diabetes mellitus (29%) or hypertension (41%). The SU.VI.MAX and Heart Protection Study will provide new information on the effectiveness of combined antioxidant treatment in populations with low and high absolute cardiovascular risk respectively.

Antioxidant treatment appears to be safe in supplementary doses, since no serious adverse effects were encountered in any of the clinical trials and, therefore, patients need not be discouraged from self-administration. Alpha-tocopherol may in addition have favourable effects in carefully selected groups who show evidence of established coronary artery disease. However, there remains insufficient evidence to support the wider use of antioxidant

therapy for prevention or treatment of cardiovascular disease in unselected groups. Effectiveness of combined antioxidant supplementation is subject to ongoing evaluation, and this could offer additional opportunities for cardiovascular risk reduction in the future.

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