LEADING ARTICLE

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Antioxidant Vitamin Supplements
Update of Their Potential Benefits and Possible Risks

Simon R.J. Maxwell

Clinical Pharmacology Unit, University of Edinburgh, Western General Hospital, Edinburgh, Scotland

Abstract

Oxidative damage to biological structures has been implicated in the pathophysiology of cardiovascular disease and cancer, the 2 most common causes of death in developed countries. This has stimulated interest in the possible role of natural antioxidant vitamins in preventing the development of these diseases. Epidemiological studies have offered support for the notion that high blood concentrations or dietary intake of antioxidant vitamins may have a protective effect. On the basis of these findings and powerful marketing strategies, many healthy members of the population are now voluntarily consuming antioxidant supplements. A number of long term, prospective, randomised, placebo-controlled trials examining the protective effect of antioxidant supplements have now been completed. Their results have been generally disappointing and have provided little evidence of efficacy. Of greater concern, they have unexpectedly raised concerns that antioxidants, notably betacarotene, might increase the rate of development of cancers in high risk individuals. For this reason regular consumption of antioxidant vitamins supplements cannot yet be advocated as a healthy lifestyle trait.

The role of free radical oxidative damage in the pathophysiology of human disease remains a topic of considerable interest to medical scientists. Free radical activity has been implicated in a variety of clinical conditions (table I) based mainly on the detection of products of oxidative damage to biomolecules including proteins, lipids and DNA. Even in healthy individuals, aerobic metabolism exposes the human body to a significant oxidative threat. Natural antioxidant mechanisms exist to prevent or retard oxidation and have been found to be defective in many of the same disease states. The popular belief that chronic degenerative human diseases may be attributable to an imbalance of oxidative stresses and endogenous antioxidant defence[1] has stimulated interest in the possibility that supplementing endogenous antioxidant defences might prevent or slow the progression of such diseases. Nowhere has this interest been greater than in the 2 commonest causes of mortality in the developed world, cardiovascular disease and cancer.

When a basic understanding of disease pathophysiology and data from epidemiological cohort studies is combined with widespread publicity, powerful marketing strategies and fears of dietary inadequacy, it is perhaps not surprising that supplements of natural antioxidant vitamins such as ascorbic acid (vitamin C), tocopherol (vitamin E) and betacarotene are now widely consumed voluntarily by the populations of developed countries. In the US the number of regular users of vitamin supplements has doubled since the 1970s to nearly 40%.[2,3] Some synthetic pharmacological agents

Table I. Diseases where an imbalance of oxidative stress and antioxidant defence has been implicated in the pathophysiology

	Acute disease	Chronic disease
Cardiovascular	Ischaemia-reperfusion injury	Atherosclerosis
		Hypertension
Respiratory	Respiratory distress syndrome	Pulmonary fibrosis
Transplantation	Acute rejection	Chronic rejection
Diabetes mellitus	Acute islet failure	Microvascular disease
		Macrovascular disease
Inflammation	Acute inflammation	Chronic inflammation
	Rheumatoid arthritis	Any acute cause
	Inflammatory bowel disease	Autoimmunity
	Pancreatitis	Vasculitis
Cancer		Cancer
Renal disease	Aminoglycoside toxicity	Chronic nephron loss
Neurological disease	Trauma	Parkinson's disease
	Stroke	Amyotrophic lateral sclerosis
		Alzheimer's disease
Critical care medicine	Trauma/sepsis	
Ocular disease	Retrolental fibroplasia	Cataract
		Senile macular degeneration
Drug toxicity	Paracetamol (acetaminophen) [overdose]	Chronic adverse effects
	Carbon tetrachloride	Alcohol
	Paraquat	Doxorubicin
Physiological	Acute exercise	Chronic exercise training
		Aging

are also marketed to doctors with the lure of the possession of antioxidant activity.

In order to be effective in preventing chronic diseases it seems that, at the very least, these agents will need to be used for prolonged periods, perhaps in high doses. This prospect should raise some concerns about their safety and potential toxicity. However, because they are natural dietary constituents they have been considered as nutrients (not drugs) thereby escaping some of the more stringent toxicity monitoring applied to pharmaceutical agents by regulatory authorities. The term 'nutriceutical' has been coined to describe their unique status.[4]

Many individuals may take natural antioxidant supplements on the basis that they provide some protection against serious disease and do no harm. Some might also have considered that at worst they provide no protection but, at least, do no harm. Largely as a result of their label as 'natural' few have given much credence to the 2 other scenarios for any therapeutic intervention: that they provide some protection but at the cost of some harm and, worst of all, that they provide no protection and do some harm.

This article briefly reviews the evidence that is currently available to support the view that antioxidant supplements prevent human disease but will also give careful consideration to the alternative hypothesis that antioxidant supplements might pose a risk to health.

1. Antioxidant Therapy for Human Disease

There are now many human diseases in which free radical oxidative damage has been implicated in the pathophysiology (table I). In some cases the oxidative stress is an acute transient phenomenon but in others the pathology probably represents the cumulative effects of many years of imbalance of oxidative stress and antioxidant defence. The most notable examples of these age-related degenerative diseases are cardiovascular disease and cancer

which account for the majority of the morbidity and mortality in developed populations.

1.1 Cardiovascular Disease

Most cardiovascular disease events occur as a result of thrombosis in a diseased atherosclerotic blood vessel. Oxidative modification of low density lipoprotein particles seems to be a critical event in their deposition in the vascular wall and the subsequent development of the atherosclerotic plaque.[5] Patients with vascular disease have low density lipoprotein particles that are more susceptible to oxidation than matched controls.[6] Tocopherol is the natural defence of the low density lipoprotein against oxidation and low density lipoprotein supplemented with tocopherol is more resistant to oxidation *in vitro*.[7]

Impaired nitric oxide–mediated endothelium-dependent vasodilatation is also a hallmark of atherosclerotic vessels and conditions such as hypertension and diabetes mellitus that are major cardiovascular risk factors. Increased local oxidative destruction of nitric oxide by free radicals may explain this dysfunction which can be partially corrected by antioxidant therapy.[8] Antioxidants may also have a beneficial impact on the development of vascular events by other mechanisms such as reducing platelet activity and the tendency to thrombus formation.[9] Recent evidence from clinical studies suggests that a high intake of antioxidants may slow the rate of atheroma progression in humans.[10]

Epidemiological surveys have linked low plasma levels or dietary intake of antioxidant vitamins (ascorbic acid, tocopherol and betacarotene) with ischaemic heart disease.[11-13] Impaired antioxidant status has also been documented in patients with hypertension and diabetes mellitus.[14,15] Two large studies in the US provided particularly compelling evidence for the protective effects of tocopherol.[16,17] The relative risk (RR) of ischaemic heart disease in cohorts of 39 910 male healthcare workers and 87 245 female nurses was 0.64 [95% confidence interval (CI) 0.49 to 0.83] and 0.59 (95% CI 0.38 to 0.91) respectively in those taking tocopherol supplements. However, since most of these studies are observational or retrospective they may be subject to selection bias. In particular, the results may be confounded by the baseline health and health awareness of the users compared with controls. Such criticisms can only be overcome by testing the benefits of antioxidants in long term prospective randomised controlled trials.

Several trials of antioxidants in populations at high risk for cardiovascular disease are currently under way but 1 small prospective study has been reported. In the Cambridge Heart Antioxidant Study (CHAOS), 2002 patients with angiographically proven coronary atherosclerosis were randomised to receive daily tocopherol 400 or 800IU or placebo for a median duration of 510 days.[18] The results of the study showed that the RR of nonfatal myocardial infarction was dramatically reduced in the tocopherol group (0.23, 95% CI 0.11 to 0.47) although this highly encouraging outcome was not reflected in any change in overall cardiovascular mortality (1.18, 95% CI 0.62 to 2.27). A more recent report has suggested that the benefit of tocopherol upon long term mortality in the CHAOS might have been underestimated due to noncompliance[19] although this retrospective reanalysis must be viewed with considerable caution.

The large Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) trial also failed to show any protection against coronary mortality among a group of male smokers given tocopherol 50 mg/day (see section 3.1 and table II for details of the study protocol).[20] Furthermore, there was no evidence of any benefit of tocopherol in protecting against cardiovascular events among the high risk subgroups of men with either a previous myocardial infarction[21] or angina pectoris.[22] Indeed, there were significantly more deaths from fatal coronary artery disease in recipients of betacarotene alone (1.75, 95% CI 1.16 to 2.64) and betacarotene with tocopherol (1.58, 95% CI 1.05 to 2.40) when compared with recipients of placebo.[21]

Table II. Results of long term antioxidant supplementation trials

Trial details (name, patients, dosage and duration)	Cancer [RR (95% CI)]	CVS death [RR (95% CI)]	Total mortality [RR (95% CI)]
Tocopherol (vitamin E)			
ATBC trial:[20] 14 564 male Finnish smokers received 50 mg/day for a median of 6.1y (range 5-8y)	0.98[a] (0.86-1.12)	0.98	1.02 (0.95-1.09)
Betacarotene			
Physicians' Health Study:[23] 22 071 US physicians received 50mg on alternate days for an average of 12y	0.98 (0.91-1.06)	1.09 (0.93-1.27)	1.02 (0.93-1.12)
CARET Study:[30] 18 314 smokers/ex-smokers/individuals with asbestos exposure received 30 mg/day plus retinol (vitamin A) 25 000 IU/day for a mean of 4y[30]	1.28[a] (1.04-1.57)	1.26 (0.99-1.61)	1.17 (1.03-1.33)
ATBC trial:[20] 14 560 male Finnish smokers received 20 mg/day for a median of 6.1y (range 5-8y)[20]	1.18[a] (1.03-1.36)	1.11	1.08 (1.01-1.16)

a Lung cancer only.

ATBC = Alpha-Tocopherol and Beta-Carotene Cancer Prevention Study; **CARET** = Carotene and Retinol Efficacy Trial; **CVS** = cardiovascular system.

The Physicians' Health Study examined the effect of betacarotene (50mg on alternate days) among 22 071 male physicians aged 40 to 84 years of age in the US (table II).[23] After an average of 12 years of treatment and follow-up there were no significant differences in cardiovascular disease events (myocardial infarction or stroke) between the placebo and betacarotene groups.

In summary, the results of the studies so far have been disappointing and have failed to substantiate any cardiovascular benefits of tocopherol in spite of its theoretical role in retarding low density lipoprotein oxidation and stabilising coronary plaques. Indeed, the general trend towards increased cardiovascular mortality has opened a serious debate about the safety of tocopherol supplementation in high risk individuals.[24] Further evidence about the efficacy of antioxidant vitamins is awaited from other ongoing trials of tocopherol alone or in combination with other antioxidants, e.g. the Heart Outcomes Prevention Evaluation (HOPE) study, the US Women's Health Study, the UK Heart Protection study and the Gruppo Italiano per lo Studio della Sopravivenza nell'infarto (GISSI) Prevention Trial.

1.2 Cancer

Although the earliest stages in the development of a neoplasm remain incompletely understood it seems that in many cases it involves mutations in nuclear DNA sequences. A variety of chemicals including free radicals are known to be mutagenic and environmental exposure to these chemicals, most notably in people who smoke, is associated with increased rates of neoplasia. Cigarette smoke is a rich source of free radical oxidants that induce DNA damage *in vitro* which can be prevented by antioxidants.[25]

It has been recognised for many years from epidemiological studies that diets rich in vegetables and fruits are associated with a reduced incidence of cancer in humans,[26,27] particularly lung cancer.[28] Although the individual dietary constituents responsible for this epidemiological effect are not known antioxidant vitamins, particularly betacarotene, have received considerable attention.[29] As noted previously, such studies are subject to selection bias and confounding factors and so confirmation of the beneficial impact of individual vitamins in prospective controlled studies is necessary.

Several trials of antioxidant therapy, mostly involving betacarotene, have already been completed in cohorts of individuals at high risk for the development of cancer. The recent ATBC trial[20] involving treatment with betacarotene and tocopherol, both alone and in combination in male smokers (see section 3.1 and table II for details of the study protocol) failed to show any protective effect of

betacarotene. In fact, it showed that there were significantly more new cases of lung cancer in the group treated with betacarotene compared with placebo recipients. Those who received tocopherol had a reduced rate of prostate and colon cancer although these were not prespecified hypotheses of the study.

The ATBC trial was followed shortly by the publication of the Carotene and Retinol Efficacy Trial (CARET) which examined the impact of treatment with a combination of betacarotene and retinol (vitamin A) or placebo on the incidence of lung cancer among smokers and workers exposed to asbestos (see section 3.2 and table II for details of the study protocol).[30] The study was stopped prematurely by the safety monitoring committee as a result of an apparently increased RR of lung cancer in the antioxidant group.

The Physicians' Health Study found no protective effect of betacarotene against cancer after an average follow-up of 12 years.[23] Similarly, betacarotene was ineffective in preventing skin cancer and a combination of betacarotene, ascorbic acid and tocopherol was ineffective in preventing colorectal adenoma formation in high risk groups.[31,32]

The Chinese Cancer Prevention Study involved 29 584 adults aged 40 to 69 years in Linxian County, China where there is a known high incidence of oesophageal and gastric cancer and low intake of several micronutrients.[33] The factorial design allowed for the testing of 4 nutritional supplement combinations one of which included betacarotene, tocopherol and selenium. During 5 years of follow-up 2127 deaths occurred of which 32% were due to either oesophageal or gastric cancer. There was a significant reduction in total mortality (RR 0.91, 95% CI 0.84 to 0.99, p = 0.03) among those receiving the above combination which was due to lower cancer rates (RR 0.87, 95% CI 0.75 to 1.00) and a lower stomach cancer rate (RR 0.79, 95% CI 0.64 to 0.99) in particular.

Selenium is an essential trace element and cofactor for glutathione peroxidase, a pivotal antioxidant enzyme that detoxifies hydrogen peroxide and lipid hydroperoxides generated by free radical oxidation. The association between a low blood level of selenium and risk of various cancers in epidemiological studies appeared to offer some promise of benefit from selenium supplementation.[34] In a recent study, 1312 male patients with a history of basal and squamous skin carcinoma living in areas of low soil selenium content were randomised to receive selenium 200 μg/day or placebo.[35] Over a mean follow-up of 4.5 years the RR of a diagnosis of prostate cancer was 0.35 (95% CI 0.18 to 0.65, p = 0.001) in those receiving selenium. Another study suggested that toenail selenium content, a marker of long term selenium intake, showed a similarly strong inverse association with development of advanced prostate cancer.[36] The ATBC trial also showed a significantly reduced incidence of prostate cancer among participants given tocopherol supplements.[20] Prostate cancer was not the primary end-point in either trial but further prospective randomised trials have been designed to test the specific hypothesis that antioxidant supplements may be protective.

The relatively disappointing results should serve to temper the enthusiasm of an increasingly health-conscious public to take antioxidant supplements as a panacea, for a healthy lifestyle and the prevention of chronic disease. Further evidence about any benefit should be available over the next few years. Until then the case for antioxidants should be considered as 'not yet proven'. The development of such diseases is a long term process and any successful antioxidant therapy will require regular use over many years. Such an intervention needs to be demonstrated unequivocally to be safe.

2. Adverse Effects of Antioxidant Supplements

Since vitamin supplements are classified as nutrients by many drug regulatory authorities many have not been subjected to the same rigorous and systematic toxicity studies that would be expected for a new pharmaceutical agent. Much of the following data are drawn from small studies of relatively short duration as well as from case reports.

2.1 Tocopherol (Vitamin E)

As a result of favourable publicity, approximately 20% of US population now take tocopherol supplements.[37] Tocopherol is a fat-soluble vitamin whose richest dietary sources are vegetable oils and it requires bile for absorption.[38] 25% of an oral dose of tocopherol is absorbed and appears in the lymph although the bioavailability varies with different preparations. Tocopherol has no specific carrier protein and is distributed among the circulating plasma lipoproteins. The lipoprotein content of tocopherol is in equilibrium with the cellular membranes of body tissues most notably adipose tissue, liver and muscle. The majority of the vitamin is eliminated unchanged in the bile.

Toxicity studies in rats give some reassurance of its lack of serious acute toxicity or teratogenicity. A number of small, short term, prospective clinical trials (some uncontrolled) of tocopherol in humans have been performed. These have used various formulations and dosages from 100 to 3200 IU/day (1 international unit is equivalent to 1mg of *dl*-α-tocopheryl acetate) and have not demonstrated any significant adverse effects.[39] However, it did become apparent that tocopherol has the potential to aggravate the anticoagulant effect of warfarin therapy.[40] A further review of the literature suggested that tocopherol could also impair coagulation in individuals with vitamin K deficiency for other reasons such as malabsorption syndromes but had no effect in vitamin K–replete individuals even at high intakes.[41] The mechanism underlying the coagulopathy is probably related to inhibition of a vitamin K–dependent carboxylase which can be overcome if adequate vitamin K is available.[42,43] Tocopherol also has the potential to impair haemostasis via a well documented antiplatelet effect.[9,44] In this regard it is interesting that tocopherol administration to premature neonates has been linked with an increased incidence of retinal and intraventricular haemorrhages.[45] Similarly, a large prospective trial has also reported an increase in fatal cerebral haemorrhagic events in patients taking tocopherol supplements.[20]

Intravenous tocopherol infusion was used widely in the 1980s for premature neonates weighing less than 1500g and was associated with severe reversible hepatotoxicity.[46] A similar effect was not seen with the oral preparation used for neonates with retrolental fibroplasia.[47] Neonates weighing less than 1500g exposed to intravenous and oral doses of tocopherol have also been reported to experience an increase in the incidence of necrotising enterocolitis.[48] Many cases of thrombophlebitis in adults were reported in 1 medical practice but no other reports have been published.[49] Gastrointestinal upset including diarrhoea and abdominal cramps were noted at a dosage of 3200 IU/day.[50] Numerous other adverse effects have been recorded in all age groups, but mainly in adults, in case reports although these must be regarded with some suspicion (table III).[51]

Most of the above studies were of limited duration (weeks) and involved relatively small numbers of participants and so would be unable to detect more chronic toxicity particularly with respect to uncommon events. A few large prospective studies of long term tocopherol supplementation have now been completed. In CHAOS (tocopherol dosage 400 to 800 IU/day), only 0.55% of patients discontinued the study medication because of adverse effects such as diarrhoea, dyspepsia or rash and there was no significant difference between groups (tocopherol *vs* placebo: RR 1.12, 95% CI 0.34 to 3.69; $p = 0.85$).[18] In the tocopherol arm of the ATBC trial (tocopherol dosage 50 mg/day), no significant adverse effects were reported other than a small increase in haemorrhagic strokes.[20]

It can be concluded that tocopherol at doses of up to 500 IU/day is nontoxic and poses negligible risk in healthy humans;[28,39,41] if this were not the case then the frequency of adverse events associated with tocopherol usage in the general population would undoubtedly be higher. Animal studies give considerable reassurance that tocopherol is not carcinogenic, mutagenic or teratogenic.[52,53] Tocopherol clearly has the potential to interfere with haemostasis although this effect is probably restricted to a cohort of susceptible individuals

Table III. Clinical and theoretical adverse effects of tocopherol (vitamin E), ascorbic acid (vitamin C) and betacarotene in humans[51]

Tocopherol
• Clinical trials
Necrotising enterocolitis – neonates <1500g
Sepsis (impaired leucocyte function) – neonates <1500g
Coagulopathy
Disturbance of prothrombin time in adults receiving warfarin
Retinal and intraventricular haemorrhage in premature neonates
Gastrointestinal tract disturbance (3200 IU/day)
• Case reports
Hepatotoxicity – intravenous preparation in neonates
Also: thrombophlebitis, breast soreness, elevated creatine kinase levels, psychiatric disturbance, pulmonary embolism, hypertension, headache, dizziness, visual complaints, hypoglycaemia, stomatitis, urticaria, aggravation of angina pectoris, aggravation of diabetes mellitus, reproductive disturbances
Ascorbic acid
• Clinical trials
Iron absorption increased
Leucocyte count increased/decreased bactericidal activity
• Case reports
Gastrointestinal symptoms including vomiting, diarrhoea and abdominal cramps (>2 g/day)
Renal stones (oxalate, urate)
Deep vein thrombosis, oesophagitis, diarrhoea, haemolysis in glucose-6-phosphate dehydrogenase deficiency
Drug interactions, e.g. warfarin, aspirin (acetylsalicylic acid), aminophylline, ethinyloestradiol
Laboratory test interactions, e.g. uric acid, glucose
Betacarotene
• Clinical trials
Hypercarotenaemia
Increased incidence of lung cancer in smokers after prolonged exposure

with borderline or impaired vitamin K activity. However, it remains a possibility that if enough such persons are entered into a prospective trial cohort over a prolonged period the consequences may become apparent. Further evidence on this point is awaited from ongoing trials.

2.2 Ascorbic Acid (Vitamin C)

Ascorbic acid (vitamin C) is absorbed from the upper intestine by an active sodium-dependent carrier-mediated saturable transport system. Tissue uptake is also via saturable active transport. The water-soluble ascorbic acid is readily eliminated unchanged or as metabolites from the body through the kidneys and so it is difficult to load the body even with high intakes. Hence, the body pool is fairly constant at about 20 mg/kg in humans even at high dietary intakes.[54] The amount of ascorbic acid filtered is small and active tubular reabsorption is almost complete.[55] Conversely, excess ascorbic acid is efficiently excreted either unchanged or as a range of metabolites including oxalate.

It has been suggested in some case reports that humans consuming large quantities of ascorbic acid may be prone to the development of oxalate stones although this usually involved intravenous administration or patients with chronic renal failure.[56] In fact, the vast majority of any excess ascorbic acid intake is excreted unchanged.[57] Some confusion about the propensity of ascorbic acid to precipitate oxalate stone formation may have arisen from a failure to prevent its oxidation to oxalate in collected urine samples. Indeed, a recent prospective epidemiological study found the risk of oxalate stones to be decreased for men con-

suming more than 1500 mg/day of ascorbic acid in comparison with less than 250 mg/day.[58] It has also been suggested that ascorbic acid may increase urate excretion although this suggestion appears also to be unfounded.[59,60] Ascorbic acid supplementation has been linked to increased mobilisation of calcium and phosphate from the skeleton in animals,[61] but the relevance of this finding in humans is unclear.

It has been postulated that ascorbic acid supplements might increase intestinal iron absorption leading to iron overload, a situation that might theoretically be associated with an increased the risk of heart disease.[62] Ascorbic acid does indeed enhance the intestinal absorbtion of nonhaem iron with maximal enhancement at approximately 25 to 50mg of ascorbic acid per meal.[63] However, ingestion of ascorbic acid 2 g/day for 2 years had no effect on serum ferritin levels suggesting that there is little impact of ascorbate on total body iron stores.[64] A meta-analysis including 1412 participants from 24 studies suggested that ascorbic acid did not increase the susceptibility to iron overload in healthy individuals.[65]

Administration of ascorbic acid 2 g/day over 15 days caused a decrease in bactericidal activity.[66] Ascorbic acid may also precipitate cyanocobalamin (vitamin B12) deficiency in patients with marginal status for other reasons.[67] Very large doses (several grams) of ascorbic acid have been reported to shorten the prothrombin time in patients treated with warfarin although no such interaction was noted in those taking only 1 g/day.[68] Ascorbic acid has also been reported to interact with a variety of other drugs and interfere with some standard laboratory tests (table III).

Several reports have suggested that ascorbic acid added to cells in culture leads to an increased rate of mutagenesis possibly secondary to increased damage to DNA.[69,70] These effects seem to be particularly likely in cultures with added Cu^{++} or Fe^{+++} ions where the interaction with the antioxidant ascorbic acid might be expected paradoxically to increase the generation of oxygen free radicals (by stimulating the Fenton reaction). It is not known whether this theoretical mechanism has any relevance *in vivo* where efficient metal ion sequestration occurs. *In vivo* tests have failed to show a significant mutagenic or carcinogenic effect.[71,72] Interestingly, a recent report in humans has suggested that high dose ascorbic acid supplementation might be associated with the increased appearance of products of DNA damage.[73]

It can be concluded that, using any standard criteria, ascorbic acid at dosages of 500 mg/day or less poses negligible risk to health.[69,72] The numerous theoretical effects of ascorbic acid have not been associated with any significant clinical events. Very high ascorbic acid intakes (above 2 g/day) have been associated with reports of minor gastrointestinal symptoms such as nausea, abdominal cramps and diarrhoea which may be related to a simple osmotic effect.

2.3 Betacarotene

Betacarotene is one of several hundred carotenoid pigments found in photosynthetic plants and bacteria and is a precursor of retinol. Betacarotene is partly metabolised to retinol but excess amounts do not lead to hypervitaminosis A because absorption of betacarotene is less efficient than retinol and the rate of conversion is too slow.[74] Betacarotene is stored predominantly in the adipose tissue and not the liver, so hepatotoxic reactions are rare. Betacarotene is used widely in the food, cosmetic and pharmaceutical industries as a colourant and so has been subjected to extensive toxicity studies *in vitro* and in animals. On the basis of its lack of mutagenicity, teratogenicity and carcinogenicity betacarotene has been classified by the US Food and Drug Administration as safe.[75]

Betacarotene is employed therapeutically in the treatment of patients with genetically inherited photosensitivities and in this context large doses of betacarotene appear to be free from toxic adverse effects.[76] Hypercarotenaemia is a recognised complication in persons taking greater than 30 mg/day over an extended period although the condition is entirely benign and reversible following discontinuation. Such persons may develop yel-

lowing of the skin (pronounced in skin folds) and an orange discolouration of the plasma but no colouration of the sclerae. Betacarotene in doses of 30 to 180 mg/day over 15 years had no other apparent adverse effects.[77] Betacarotene does not appear to pose a risk for the developing fetus.[78] Unsubstantiated anecdotal reports of leucopenia, reproductive disorders, prostate cancer, retinopathy and allergic reactions[79] have been made in persons taking large amounts of betacarotene although these have not been substantiated in any clinical trials.

One short term phase I toxicity trial of betacarotene in doses up to 60 mg/day given over a 9-month period reported a dose-related fall in tocopherol level.[80] This raised the possibility that there was a kinetic or oxidative interaction between the 2 antioxidant nutrients. However, further large studies failed to confirm this effect[81-83] and even suggested a small but significant increase in tocopherol level in response to betacarotene.[30]

It can be concluded that in healthy individuals betacarotene supplements pose negligible risk.[84] Following the publication of randomised controlled trials in older populations at higher risk of malignant disease some concerns have now been raised about the potential for the long term use of betacarotene supplements to increase lung cancers.[20,30] These issues will be addressed in section 3.

2.4 Retinol (Vitamin A)

Retinol is not strictly an antioxidant but is often included in multi-antioxidant preparations. In contrast to the above vitamins there are well described acute and chronic toxicity syndromes associated with retinol.[51,77,79,85] Consumption of daily supplements of 7.5mg of preformed retinol equivalents (25 000 IU) taken for 6 years have been associated with the development of liver cirrhosis.[86] There have also been reports of an increased risk of neural crest defects in babies of mothers ingesting more than 3 mg/day retinol equivalents (more than 10 000 IU) [average 6.5mg].[87] Another study suggested a characteristic defect associated with an intake of 5.4mg retinol equivalents/day.[88] A recently reported study of supplementation with 7 mg/wk retinol equivalents and 42 mg/wk of betacarotene among 44 646 women of childbearing age in Nepal reported no significant adverse effects of treatment.[89] In summary, there is no evidence that supplements of up to 3mg retinol equivalents/day are harmful to healthy adults although the vitamin is probable best avoided in expectant mothers. There is no evidence that betacarotene contributes to retinol toxicity, even when betacarotene is ingested in large amounts.[90]

3. Could Long Term Use of Antioxidant Supplements be Harmful?

The hypothesis-generating evidence from laboratory and epidemiological studies led to several large prospective randomised controlled trials of antioxidant supplements. Although their main aim was to establish the efficacy of antioxidants in disease prevention they were also well positioned to examine any potential adverse outcomes. Two recent studies have given particular cause for concern (table II).

3.1 The Alpha-Tocopherol and Beta-Carotene Cancer Prevention Study

In the ATBC trial, 29 133 male smokers aged 50 to 69 years of age were randomised to receive either tocopherol 50 mg/day alone, betacarotene 20 mg/day alone, tocopherol 50 mg/day plus betacarotene 20 mg/day or placebo in a 2×2 factorial design. The participants were followed for 5 to 8 years (median 6.1 years).[20] The median vitamin concentrations were significantly raised at 3 years in the supplemented groups compared with baseline: tocopherol from 26.7 to 40.2 μmol/L (1.5-fold increase) and betacarotene from 0.32 to 5.59 μmol/L (17.5-fold increase). New diagnoses of lung cancer (+18%, 95% CI +3 to +36%, $p = 0.01$) and total mortality (+8%, 95% CI +1 to +16%, $p = 0.02$) were significantly increased amongst the group who received betacarotene compared with those who did not. The increase in total mortality with betacarotene was attributable to a combina-

tion of causes including lung cancer, ischaemic heart disease and ischaemic or haemorrhagic strokes. Tocopherol had no impact on either new diagnoses of lung cancer (–2%, 95% CI –14 to +12%, p = 0.8) or total mortality (+2%, 95% CI –5 to +9%, p = 0.6). The neutral effect on mortality was made up of a reduction in deaths due to ischaemic heart disease or ischaemic stroke and an increase in deaths from nonlung cancers and haemorrhagic strokes. There was no evidence of any interaction between betacarotene and tocopherol with respect to the incidence of lung cancer during the trial.

An interesting observation made in the trial was that when the whole trial cohort was divided into quartiles according to baseline presupplementation vitamin concentration those in the lowest quartiles had a significantly increased incidence of lung cancer compared with the highest (incidence per 10 000 person-years 56.8 *vs* 41.8 for tocopherol and 53.3 *vs* 43.1 for betacarotene). Moreover, a similar inverse correlation was noted for dietary vitamin intake recorded at baseline (61.4 *vs* 40.6 for tocopherol and 47.9 *vs* 39.9 for betacarotene). This substantiates the belief that a major contribution to the likelihood of developing lung cancer was made by pretrial dietary behaviour with regard to the index vitamins or closely associated dietary constituents rather than the assigned supplementation group.

3.2 The Carotene and Retinol Efficacy Trial

CARET examined the impact of a combination of betacarotene 30 mg/day and retinol 25 000 IU/day or placebo on the incidence of lung cancer among 18 314 American smokers, ex-smokers and workers exposed to asbestos.[30] The study was stopped 21 months earlier than originally planned after an average follow-up of 4 years. At that stage the active treatment group had a RR of lung cancer of 1.28 (95% CI, 1.04 to 1.57; p = 0.02). There was also an increase in RR of death from any cause (1.17, 95% CI 1.03 to 1.33), death from lung cancer (1.46, 95% CI 1.07 to 2.00) and death from cardiovascular disease (1.26, 95% CI 0.99 to 1.61).

The reasons for the possible deleterious effects of the antioxidant vitamins are still a matter of debate. The unexpected results of the 2 trials have been re-analysed to examine which groups were prone to an adverse outcome. In the ATBC study, betacarotene did not have an adverse impact in light smokers (5 to 19 cigarettes/day) who had a RR of 0.97 (nonsignificant).[91] In the CARET study, former smokers with no asbestos exposure who were given betacarotene and retinol had a decreased RR of lung cancer (0.80, nonsignificant) although even this group had increased total mortality.[92]

Both analyses imply that smoke exposure is a prerequisite for an adverse effect of betacarotene. This has some biological plausibility since *in vitro* studies show that antioxidants may have pro-oxidant effects when combined with some oxidants.[93] The extremely high betacarotene concentrations obtained in both studies may conceivably interact with cigarette smoke to create a pro-oxidative environment.[94] It has also been suggested that betacarotene may act as a promoter of existing latent tumours rather than an initiator. The fact that individuals with the highest presupplementation betacarotene concentrations had the lowest lung cancer rates will lead to speculation that more moderate concentrations of betacarotene are indeed effective but that the increase in betacarotene intake in these studies was simply too aggressive. A less optimistic interpretation is that betacarotene is merely a surrogate marker for other protective dietary constituents. Higher alcohol intakes also seemed to be associated with the harmful effects of betacarotene supplementation although a convincing dose-response relationship was only seen in ATBC.[91,92] Alcohol has been postulated to interact with retinol in the liver.[95]

Three other studies are more reassuring about the impact of betacarotene. At entry into the Physicians' Health Study[23] (table II) in 1982, 11% of the study participants were current smokers and 39% were ex-smokers. After an average of 12 years of treatment and follow-up there were no significant differences between the placebo and beta-

carotene groups with respect to the incidence of or death resulting from malignant neoplasms or cardiovascular disease (myocardial infarction or stroke). No increase in lung cancer risk was seen among patients randomised to betacarotene in 2 other shorter supplementation studies examining the prevention of skin and colorectal cancer.[31,32]

The results of these studies seem to eliminate the possibility that high dose betacarotene supplements have any beneficial impact on the development of cancer. Furthermore, the ATBC and CARET studies suggest that betacarotene is best avoided by regular smokers although there does not seem to be a need for concern about its use by otherwise healthy individuals. This unexpected outcome should reaffirm the fact that promising basic mechanistic research and epidemiology should be underpinned by prospective randomised controlled trials before any definitive conclusions are drawn. Many members of the public probably consider that natural antioxidants, in particular, are free of harmful effects and are a reasonable gamble as a health promoter. This view is probably naive. In keeping with most other therapeutic interventions it seems that even with natural vitamins there may be a cost-benefit analysis to consider.

4. Conclusion

Basic research has pointed to the importance of oxidative mechanism in the development of both atherosclerotic cardiovascular disease and cancer. Several large epidemiological surveys supported the hypothesis that exposure to natural antioxidant vitamins might retard the development of both conditions. Disappointingly, there remains little evidence from properly conducted randomised controlled trials for a beneficial effect of antioxidant supplements on either condition. Factors that might account for the discrepancy between observational studies and randomised trials include self-selection, uncontrolled confounding, association of antioxidant intake in epidemiological studies with other unidentified but efficacious dietary factors (e.g. minerals, flavonoids, carotenoids other than betacarotene) and use of inappropriate antioxidant doses in the clinical trials. Nevertheless, powerful marketing and intense popular interest have led to a massive increase in the proportions of healthy individuals who take antioxidant supplements in the hope that some health benefit exists and the expectation that there is little associated risk.

In the short and medium term typical supplementary doses of tocopherol, ascorbic acid or betacarotene do not appear to be associated with any significant adverse effects. However, there is now evidence that long term betacarotene usage may be associated with an adverse impact on major end-points such as lung cancer particularly among unhealthy high risk individuals. The reasons are unclear but the fact that its concentrations were so greatly increased in the setting of an already markedly pro-oxidant environment may be relevant. There is some evidence from physiological studies that antioxidants may have a biphasic effect: low doses (similar to those available from dietary sources) may be beneficial while high doses of antioxidant compounds may act as pro-oxidants.[73,96] Injudicious use of antioxidants may inadvertently accelerate rather than retard disease processes. Enthusiastic endorsement of antioxidants should now be tempered until further trial evidence is available. Healthcare professionals should continue to promote proven healthy lifestyle traits and emphasise that supplements are unlikely to be a convenient short cut. This cautious approach was recently endorsed in the recommendations of the Nutrition Committee of the American Heart Association.[97]

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Correspondence and reprints: Dr *Simon R.J. Maxwell,* Clinical Pharmacology Unit, University of Edinburgh, Western General Hospital, Porterfield Road, Edinburgh EH4 2LH, Scotland.