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A review of the epidemiological evidence for the 'antioxidant hypothesis'

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Abstract

Objective: The British Nutrition Foundation was recently commissioned by the Food Standards Agency to conduct a review of the government's research programme on *Antioxidants in Food*. Part of this work involved an independent review of the scientific literature on the role of antioxidants in chronic disease prevention, which is presented in this paper.

Background: There is consistent evidence that diets rich in fruit and vegetables and other plant foods are associated with moderately lower overall mortality rates and lower death rates from cardiovascular disease and some types of cancer. The 'antioxidant hypothesis' proposes that vitamin C, vitamin E, carotenoids and other antioxidant nutrients afford protection against chronic diseases by decreasing oxidative damage.

Results: Although scientific rationale and observational studies have been convincing, randomised primary and secondary intervention trials have failed to show any consistent benefit from the use of antioxidant supplements on cardiovascular disease or cancer risk, with some trials even suggesting possible harm in certain subgroups. These trials have usually involved the administration of single antioxidant nutrients given at relatively high doses. The results of trials investigating the effect of a balanced combination of antioxidants at levels achievable by diet are awaited.

Conclusion: The suggestion that antioxidant supplements can prevent chronic diseases has not been proved or consistently supported by the findings of published intervention trials. Further evidence regarding the efficacy, safety and appropriate dosage of antioxidants in relation to chronic disease is needed. The most prudent public health advice remains to increase the consumption of plant foods, as such dietary patterns are associated with reduced risk of chronic disease.

Keywords
Antioxidant
Fruit and vegetables
Oxidative damage
Cancer
Cardiovascular disease

In 2001, the British Nutrition Foundation was commissioned by the Food Standards Agency (FSA) to conduct a review of the government's research programme on *Antioxidants in Food*, which the Agency inherited from the Ministry of Agriculture, Fisheries and Food. The objective of the review was to set the findings of the government-funded research projects in an international context and to make recommendations about the future of the programme (the full report of this review is held in the FSA library). Part of this work involved summarising the epidemiological evidence for the so-called 'antioxidant hypothesis', which forms the basis of this paper.

The antioxidant hypothesis

A large number of epidemiological studies have shown that people who consume a diet with a high content of fruit and vegetables and other plant foods (e.g. nuts) are at reduced risk of developing cancer and cardiovascular disease (CVD)[1,2]. Beneficial effects of such dietary patterns have also been reported for other chronic conditions. For example, inverse associations have been demonstrated between fruit and vegetable consumption and the risk of age-related macular degeneration (AMD)[3], cataract[4,5] and chronic obstructive pulmonary disease (COPD), including asthma and bronchitis[6,7]. This has led to attempts to identify the specific components responsible for the health effects of these plant foods. A popular explanation, both within the scientific community and among members of the public, is that antioxidant nutrients, including vitamin C, vitamin E, the carotenoids (e.g. β-carotene, lycopene and lutein), selenium and the flavonoids (e.g. quercetin, kaempferol, myricetin, luteolin and apigenin), prevent carcinogenesis and atherogenesis by interfering passively with oxidative damage to DNA, lipids and proteins. Normal oxidative metabolism produces large amounts of potentially dangerous oxidants (free radicals) that can damage cells and tissues in a number of ways: damaging

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biomolecules and cell components, triggering the activation of specific signalling pathways, creating toxic products, altering gene expression and enzyme activity, and disrupting normal repair mechanisms[8]. Antioxidants prevent free-radical-induced tissue damage by preventing the formation of radicals, scavenging them or by promoting their decomposition. The disruption of the delicate balance between pro- and antioxidants is termed oxidative stress and has been implicated in the pathophysiology of many chronic diseases, including CVD, ageing, diabetes and cancer.

Antioxidants and CVD

Of all the diseases in which excess oxidative stress has been implicated, CVD has the strongest supporting evidence. Oxidation of low-density lipoprotein (LDL) cholesterol may be a key step in the development of atherosclerosis, a known risk factor in the development of CVD[9]. Oxidised LDL cholesterol is preferentially taken up by macrophages to create the foam cells characteristic of fatty streaks, which are precursors of atherosclerotic plaques. In addition, oxidised LDL cholesterol appears to be cytotoxic towards endothelial cells and to decrease the motility of tissue macrophages. Dietary antioxidants (particularly vitamin E, which is carried primarily in LDL cholesterol) may, therefore, provide protection by reducing the oxidation of LDL cholesterol and thereby slowing the development of atherosclerosis.

Although there are some exceptions, cross-sectional studies comparing different populations within one country or between different countries have found that the incidence of CVD, particularly in Europe, is inversely related to plasma levels of β-carotene, vitamin E (α-tocopherol) and, to a lesser extent, vitamin C[10,11]. Countries with a very high prevalence of CVD, such as Scotland, Northern Ireland and Finland, have significantly lower plasma levels of vitamin E and β-carotene, while the Mediterranean countries have relatively higher blood levels and a lower incidence of CVD[12]. An ecological study based on middle-aged men from 16 different cohorts showed a similar inverse association between flavonoid intake and coronary mortality[13], and increased rates of heart disease have also been reported in areas with low selenium status[14].

Some case–control studies have confirmed the same type of relationship at an individual level; for example, subjects with CVD have been shown to have lower levels of plasma vitamin E and selenium, compared with subjects without CVD[15]. Other studies have found lower leucocyte ascorbic acid concentrations in subjects with angiographic coronary artery disease compared with controls[12] and lower adipose tissue levels of β-carotene in patients with myocardial infarction (MI) compared with hospital-based controls[16]. Such studies, however, cannot exclude the possibility of alterations in nutritional status as a consequence of the disease or from subsequent lifestyle modifications.

More convincing evidence is provided by prospective studies in which nutrient status is measured years before the onset of the disease. Several large cohort studies have investigated the relationship between antioxidant nutrients in the diet or vitamin supplements and the incidence of CVD, and have generally demonstrated a trend towards decreasing risk of CVD incidence or mortality with higher dietary intake of vitamin E[17–21], β-carotene[22,23] and vitamin C[24] and with higher plasma levels of these vitamins[25–30]. Interestingly, findings of prospective studies that have investigated the role of vitamin supplements (predominantly in the USA) have been less convincing[20,21,31].

The results of prospective studies investigating the link between low selenium status/intake and heart disease have been mixed. Two of the studies that did find an association[32,33] were conducted in Finland, where selenium intake has been very low until recently. For example, Salonen *et al.* demonstrated a 3.6-fold increase in coronary deaths and a 2.7-fold increase in heart attacks amongst men who had very low serum selenium status ($<45\ \mu g\ l^{-1}$)[32]. In contrast, studies in populations with higher selenium intakes have not found an association[28,34–36]. It is, therefore, conceivable that cardiovascular risk might be influenced only by very low selenium status[37]. A diet rich in flavonoids (mainly from onions, apples, tea and wine) has been inversely associated with subsequent CVD in some, but not all, prospective studies[38].

Evidence from intervention trials

A number of large intervention trials for primary and secondary CVD prevention have now been conducted to try to demonstrate a causal relationship between vitamin C, vitamin E and β-carotene and CVD (Table 1). The most positive results came from the Cambridge Heart Antioxidant Study (CHAOS), a controlled trial on 2002 heart disease patients with angiographically proven coronary atherosclerosis, randomly assigned to receive vitamin E or an inactive placebo[51]. The trial continued for almost 2 years. Vitamin E treatment significantly reduced the risk of CVD death and non-fatal MI combined (relative risk (RR) 0.53, 95% confidence interval (CI) 0.34–0.83). However, the decrease was primarily due to a dramatic reduction in non-fatal MI and CVD deaths did not alter significantly. Other smaller trials have also demonstrated beneficial effects of antioxidant supplementation in groups of patients at high risk of oxidative stress. For example, in a trial of 196 haemodialysis patients with pre-existing CVD who received 800 IU day^{-1} of vitamin E or matching placebo and were followed for a median of 519 days, a significant reduction was found in the primary endpoint (fatal or non-fatal MI, sudden death, ischaemic stroke, peripheral vascular disease or angina) amongst those who received the antioxidant supplementation (RR 0.54, 95% CI 0.33–0.89)[57]. In another randomised,

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Table 1 Large intervention trials (>1000 subjects): antioxidants and CVD

Study	Country	Study population	Duration of treatment (years)	Daily dose	Results
PRIMARY PREVENTION					
Linxian Cancer Prevention Study[39]	China	29 584 poorly nourished men and women, 40–69 years	5.2	15 mg β-carotene, 30 mg α-tocopherol and 50 μg selenium	Non-significant ↓ in cerebrovascular mortality
Alpha Tocopherol Beta Carotene Cancer Prevention Study (ATBC)[40,41]	Finland	29 133 male cigarette smokers, 50–69 years	6.1	50 mg α-tocopherol and/or 20 mg β-carotene	11% ↑ in CHD mortality among β-carotene group 50% ↑ in haemorrhagic stroke mortality among vitamin E group 62% ↑ in intracerebral haemorrhage among β-carotene group 14% ↓ in cerebral infarction among vitamin E group
Beta Carotene and Retinol Efficacy Trial (CARET)[42]	USA	14 254 heavy smokers, 4060 asbestos workers, 45–69 years	4	30 mg β-carotene and 25 000 IU retinol	26% ↑ in CVD (NS) 17% ↑ in total mortality
Physicians' Health Study (PHS)[43]	USA	22 071 male physicians, 40–84 years	12	50 mg β-carotene and/or aspirin (alternate days)	No effect on incidence or mortality from MI or stroke
Women's Health Study[44]	USA	39 876 healthy women, ≥45 years	2.1	50 mg β-carotene (alternate days)	No effect on incidence of MI or stroke or on CVD mortality
Women's Health Study[45]	USA	39 876 healthy women, ≥45 years	Unknown	600 IU α-tocopherol and/or 100 mg aspirin (alternate days)	Effect on MI, stroke and CVD mortality awaited
Vitamin A and Cancer Prevention II[46]	Australia	1204 former asbestos workers, men and women, 40–83 years	5	30 mg β-carotene or 25 000 IU retinol (no placebo group)	No effect of β-carotene on CHD mortality
Skin Cancer Prevention Trial[47]	Australia	1720 men and women, 27–84 years, with recent non-melanoma skin cancer	4.3	50 mg β-carotene	No effect on CVD mortality
Physicians' Health Study II[23]	USA	15 000 healthy male physicians, ≥55 years	12	50 mg β-carotene, 400 IU α-tocopherol (alternate days) plus 500 mg vitamin C, multivitamin (daily)	Effects on CVD awaited
SUpplementation en VItamines et Minéraux AntioXydants (SU.VI.MAX)[48]	France	12 735 men (45–69 years) and women (35–60 years)	8	6 mg β-carotene, 30 mg α-tocopherol, 120 mg vitamin C, 100 μg selenium and 20 mg zinc	Unpublished data show no effect on CVD
*SECONDARY PREVENTION**					
ATBC[49]	Finland	1862 male heavy smokers with previous MI, 50–69 years	5.3	50 mg α-tocopherol and/or 20 mg β-carotene	Reduced non-fatal acute ischaemia (vitamin E and β-carotene) No effect on risk of MI (vitamin E) Increased risk of MI (β-carotene)
ATBC[50]	Finland	1795 male heavy smokers with previous angina pectoris, 50–69 years	4	50 mg α-tocopherol and/or 20 mg β-carotene	No effect on symptoms or progression of angina pectoris (vitamin E)
Cambridge Heart Antioxidant Study (CHAOS)[51]	UK	2002 patients with coronary atherosclerosis, mean age 62 years	1.4	400 or 800 IU α-tocopherol	77% ↓ in non-fatal MI No benefit on CVD mortality
GISSI Prevenzione Trial[52]	Italy	11 324 patients with recent MI (no defined age range)	3.5	300 mg α-tocopherol and/or 1 g *n* − 3 PUFA	No benefit from vitamin E 15% ↓ in risk of death, non-fatal MI and stroke from *n*− 3 PUFA
Heart Outcomes Prevention Evaluation Study (HOPE)[53]	Canada	9541 high-risk men and women, >55 years	4–6	400 IU α-tocopherol and/or ACE inhibitor	No effect of vitamin E on MI, stroke or CVD death

Table 1 *Continued*

Study	Country	Study population	Duration of treatment (years)	Daily dose	Results
Primary Prevention Project (PPP)[54]	Italy	4495 men and women with one or more CVD risk factors, mean age 64 years	3.6	Low-dose aspirin and/or 300 mg α-tocopherol	No effect of vitamin E on any pre-specified CVD endpoint including CVD mortality, MI and stroke. However, the study had inadequate power due to premature interruption of the trial
Women's Antioxidant Cardiovascular Study (WACS)[55]	USA	8000 women with prior CVD even or ≥ 3 coronary risk factors, ≥ 40 years	4	50 mg β-carotene (alternate days) or 600 IU α-tocopherol (alternate days) or 500 mg vitamin C (daily)	Effect on CVD awaited
Heart Protection Study[56]	UK	20 536 high-risk men and women, 40–80 years	≥ 5	20 mg β-carotene, 600 mg α-tocopherol and 250 mg vitamin C	No reduction in fatal or non-fatal MI or stroke

CVD – cardiovascular disease; GISSI – Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardio; MI – myocardial infarction; PUFA – polyunsaturated fatty acids; ACE – angiotensin-converting enzyme; CHD – coronary heart disease; NS – not significant.
* Secondary prevention is defined as including patients with known or documented vascular disease.

placebo-controlled trial of 156 men with previous coronary artery bypass graft surgery, subjects supplemented with 100 IU or more of vitamin E per day experienced less coronary artery lesion progression than subjects supplemented daily with 100 IU or less[58]. A recent study has also demonstrated vitamin E supplementation to suppress restenosis in surgically induced atherosclerosis[59].

However, other secondary prevention trials have failed to detect any benefit of vitamin E or other antioxidant nutrients[52,53,56]. The Heart Protection Study examined the effect of a cocktail of antioxidant vitamins (600 mg vitamin E, 250 mg vitamin C and 20 mg β-carotene) or placebo in over 20 000 UK adults (aged 40–80 years) with coronary heart disease, other occlusive arterial disease or diabetes mellitus, who were supplemented for 5 years[56]. Although blood levels of antioxidant vitamins were substantially increased, no significant reduction in the 5-year mortality from vascular disease or any other major outcome was noted. In the GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardio) Prevenzione Trial, dietary fish oils reduced the risk of death, non-fatal MI and stroke in subjects with recent MI, but vitamin E supplementation (300 mg daily for 3.5 years) did not provide any benefit[52].

Results of most primary prevention trials have also been unable to support the findings of the observational studies. For example, the Alpha Tocopherol Beta Carotene Prevention Study (ATBC) demonstrated no effect of β-carotene or vitamin E supplementation on the incidence of large abdominal aortic aneurysm[60] or on symptoms and progression of intermittent claudication[61] amongst men supplemented for an average of 6 years. The vitamin E supplement was associated with a reduction in cerebral infarction but a 50% increase in haemorrhagic stroke mortality[40,41]. The net effect on all strokes was a small decrease in the incidence but a moderate (although insignificant) increase in mortality[41]. Supplemental β-carotene increased the incidence of cerebral hemorrhage[41] and led to an increase in deaths from MI[40].

The published results of the SUpplementation en VItamines et Minéraux AntioXydants study (SU.VI.MAX) are still awaited. This is a randomised, double-blind, placebo-controlled, primary prevention trial in over 12 000 men and women in France, designed to test the efficacy of a cocktail of antioxidant vitamins and minerals, at nutritional doses, on premature death from CVD and cancer[48]. The study, which has been underway since 1994, involves supplementation with a combination of antioxidant nutrients (120 mg vitamin C, 30 mg vitamin E, 6 mg β-carotene, 100 μg selenium, 20 mg zinc) at doses around one to three times the daily recommended dietary allowances. Preliminary results presented at a recent scientific conference suggest that this regime has also demonstrated no effect on CVD risk (5th Congrès National de la Société Française d'Athérosclérose et de l'Arcol et de la Journée Nationale, June 2003).

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Systematic reviews and meta-analyses of the trials to date have, therefore, concluded that despite evidence from observational studies that people with a high occurrence of CVD often have low intakes or plasma levels of antioxidant nutrients, supplementation with any single antioxidant nutrient or combination of nutrients has not demonstrated any benefit for primary or secondary CVD prevention[62–65] (Fig. 1).

Antioxidants and cancer

The oxidative hypothesis of carcinogenesis asserts that carcinogens generate reactive oxygen species that damage RNA and DNA in cells, predisposing these cells to malignant changes. Oxidative damage to DNA occurs at a daily rate of 10^4 hits per cell in humans[66]. Most, but not all, damage is corrected by internal surveillance and repair systems. Antioxidants are proposed to prevent cell damage by neutralising free radicals and oxidants, thus preventing subsequent development of cancer.

Case–control and cohort studies have consistently shown an inverse association between intake of carotenoids (α-carotene, β-carotene, lutein and lycopene) and risk of lung and stomach cancer[2,67]. Comparing subjects in the highest category of dietary carotenoid intake with those in the lowest, risk reductions from 10 to 90% have been observed[68]. A number of prospective nested case–control studies have also shown an inverse association between high blood levels (usually of β-carotene) and subsequent risk of lung cancer (risk reductions of 40–80% have been reported)[68], with plasma β-carotene concentrations in the range of 0.34 to 0.53 $\mu mol\,l^{-1}$ being associated with the lowest risk[69].

Most observational studies have also reported significant inverse associations between vitamin C intake and cancer risk[70] (risk reductions comparing the highest individual intake of vitamin C with the lowest have been between 10 and 70%)[68]. The most consistent data have been with non-hormone-dependent cancer sites of the oral cavity, larynx, pharynx and oesophagus, as well as of the lung, stomach, colon and rectum. Of the hormone-dependent cancers, there is somewhat weaker evidence for a link with breast cancer[20,71] but little evidence for any protective effect in ovary or prostate cancer. Data from nested case–control studies are limited, primarily because of the deterioration of this vitamin during frozen storage. However, two large studies have investigated the association. A 12-year follow-up of the NHANES II (National Health and Nutrition Examination Survey) cohort of US adults found men in the lowest serum ascorbate quartile to have a 62% higher risk of dying from cancer than men in the highest quartile, but found no association with cancer mortality in women[72]. Similarly, the UK arm of the European Prospective Investigation into Cancer and Nutrition (EPIC) found an inverse association between plasma ascorbic acid concentration and cancer mortality in men (a rise of 20 $\mu mol\,l^{-1}$ in plasma ascorbic

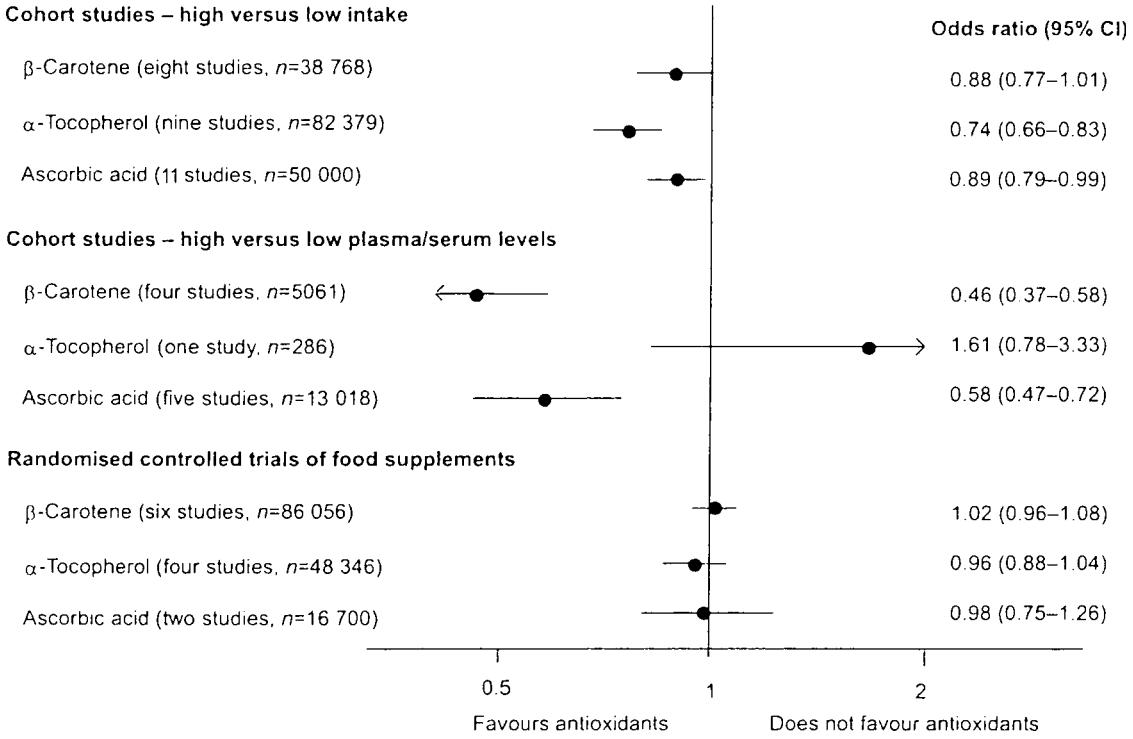

Fig. 1 A summary of the results of the cohort studies and randomised controlled trials investigating the effect of antioxidants on cardiovascular disease risk from a recent systematic review (CI, confidence interval). Reproduced from Asplund[64] (with permission from Blackwell Publishing)

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acid concentration was associated with a 21% reduction in risk, $P < 0.02$), but not women[30].

Regions and countries with low selenium intakes or status have been shown to have higher rates of cancer death[73,74], and cancer patients have also been found to have lower concentrations of selenium in case–control studies[75,76], although these findings must be interpreted with care as blood levels have been shown to decline as disease progresses and patients with advanced disease tend to have lower blood selenium concentrations[76]. Prospective studies have generally shown low selenium status to be associated with significantly increased risk of cancer incidence and/or mortality. The large Finnish Mobile Health Examination Survey showed a reduced risk of certain cancers, notably of the stomach and lung, in men[77], and other prospective studies have shown an inverse association with risk of prostate cancer[78] and colorectal adenomas[79].

In contrast, most observational studies examining the relationship between dietary intake of vitamin E and risk of developing cancer have provided little evidence for a protective role of this vitamin. Around a third of studies measuring blood levels of vitamin E have shown that those with the highest levels have a lower risk of cancer (risk reductions observed have ranged from 20 to 80%)[68], while the remainder have found no significant differences between cases and controls. Epidemiological data investigating the role of flavonoids in cancer prevention are scarcer and findings have been inconsistent. A prospective study of Finnish men and women found an inverse association between flavonoid intake and incidence of all sites of cancer combined following adjustment for many factors including intake of antioxidant vitamins[80]. This association was mainly a result of lung cancer, which presented a relative risk of 0.54 (95% CI 0.34–0.87) in the highest compared with the lowest quartile of flavonoid intake. Intake of flavonols and flavones was also inversely associated with the risk of lung cancer (RR 0.56, 95% CI 0.5–0.69 in the highest vs. lowest quartile) but unrelated with any other type of cancer in the ATBC trials[81]. However, Hertog *et al.* demonstrated no association between dietary intake of the five major flavonoids and mortality from total cancer, lung cancer, colorectal cancer or stomach cancer in an analysis of data from the Seven Countries Study after 25 years of follow-up[13] or with mortality from cancer at all sites in the Zutphen Elderly Study[82]. In a more recent analysis of the same cohort, intake of catechins was found not to be significantly associated with the incidence of epithelial or lung cancer[83]. Therefore, despite plausible mechanisms, there is little observational evidence for any beneficial effect of flavonoids against cancer.

Evidence from intervention trials

The most positive findings from the randomised controlled trials to date were from the study in Linxian (China) in a rural population with poor nutritional status, where supplementation with a combination of β-carotene, selenium and vitamin E for 5 years led to a 21% reduction in stomach cancer mortality and a 13% reduction in total cancer mortality[39] (Table 2). However, this study is unable to contribute to knowledge about the effects of individual antioxidants or offer any insight into their effects in populations with good nutritional status. There have now been a number of large, double-blind, randomised intervention trials in well-fed subjects using high-dose β-carotene supplements, either alone or in combination with other agents. The ATBC Cancer Prevention Trial randomly assigned 29 133 Finnish male smokers, aged 50–69 years, to receive 20 mg of β-carotene per day and 50 mg of α-tocopherol or placebo, using a 2×2 factorial design[40]. After 6 years of follow-up, β-carotene showed no protective effect on the incidence of any type of cancer[40,84–86]. In fact, those randomised to receive this vitamin had an 18% higher risk of lung cancer (RR 1.18, 95% CI 1.03–1.36) and an 8% higher total mortality than non-recipients. Subgroup analyses suggested that the adverse effect of β-carotene on lung cancer risk was restricted to heavy smokers[94]. This adverse effect was, however, lost on post-intervention follow-up[95]. The Beta Carotene and Retinol Efficacy Trial (CARET) was also terminated early because of similar findings; subjects receiving a combination of supplements (30 mg β-carotene and 25 000 IU vitamin A daily) experienced a 28% increased risk of lung cancer incidence (95% CI 1.07–2.00)[42]. Subgroup analyses also suggested that the effect was found in current, but not former smokers[42]. By contrast, in the Physicians' Health Study (PHS), supplementation of male physicians with 50 mg of β-carotene on alternate days had no effect on cancer incidence (men who were smokers did not experience any benefit or harm)[43]. The Women's Health Study terminated the β-carotene arm of the supplement interventions following the release of the CARET and PHS results[96]. The Heart Protection Study demonstrated no effect on 5-year cancer incidence or mortality from supplementation with 20 mg β-carotene in combination with 600 mg α-tocopherol and 250 mg vitamin C in individuals at high risk of CVD, despite increases in blood concentrations of these nutrients (plasma β-carotene concentrations rose four-fold)[56].

There is little evidence for any beneficial effects of supplementation with β-carotene for skin cancer prevention. The PHS found no effect after 12 years of β-carotene supplementation on the development of a first non-melanoma skin cancer[87]. Two smaller trials have also supported this finding. Amongst 1621 men and women followed for 4.5 years (most of whom had no history of skin cancer at baseline), those randomised to supplementation with 30 mg β-carotene did not experience any reduction in risk of basal-cell or squamous-cell carcinoma[88]. The β-carotene supplement also had no influence

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Table 2 Large intervention trials (>1000 subjects): antioxidants and cancer

Study	Country	Study population	Duration of treatment (years)	Daily dose	Results
PRIMARY PREVENTION					
Linxian Cancer Prevention Study[39]	China	29 584 poorly nourished men and women, 40–69 years	5.25	15 mg β-carotene,30 mg α-tocopherol and 50 μg selenium (also tested effect of retinol & zinc, riboflavin & niacin, vitamin C & molybdenum)	13% ↓ in cancer mortality 21% ↓ in stomach cancer (supplementation with other nutrients had no effect on cancer mortality)
Alpha Tocopherol Beta Carotene Cancer Prevention Study (ATBC)[40,84–86]	Finland	29 133 male cigarette smokers, 50–69 years	5–8	50 mg α-tocopherol and/or 20 mg β-carotene	18% ↑ in lung cancer among β-carotene group No effect of vitamin E on lung cancer 34% ↓ in incidence of prostate cancer among vitamin E group Later analysis showed no significant effects of vitamin E or β-carotene on incidence of colorectal, pancreatic or urinary tract cancers
Beta Carotene and Retinol Efficacy Trial (CARET)[42]	USA	14 254 heavy smokers, 4060 asbestos workers, 45–69 years	4	30 mg β-carotene and 25 000 IU retinol	28% ↑ in lung cancer
Physicians' Health Study (PHS)[43,87]	USA	22 071 male physicians, 40–84 years	12	50 mg β-carotene (alternate days)	No effect on incidence of malignant neoplasms or non-melanoma skin cancer
Women's Health Study[44]	USA	39 876 healthy women, without history of CVD or cancer (other than non-melanoma skin cancer), ≥45 years	2.1	50 mg β-carotene (alternate days)	No effect on incidence of cancer
The Nambour Skin Cancer Prevention Trial[88]	Australia	1621 men and women (73% without skin cancer at baseline), 20–69 years	4.5	30 mg β-carotene with or without sunscreen application	No effect on basal-cell or squamous-cell carcinoma
Physicians' Health Study II[23]	USA	15 000 healthy male physicians, ≥55 years	Unknown	50 mg β-carotene, 400 IU α-tocopherol (alternate days) plus 500 mg vitamin C, multivitamin (daily)	Effects on total and prostate cancer awaited
SUpplementation en VItamines et Minéraux AntioXydants (SU.VI.MAX)[48]	France	12 735 men (45–69 years) and women (35–60 years)	8	6 mg β-carotene, 30 mg α-tocopherol, 120 mg vitamin C, 100 μg selenium and 20 mg zinc	Early reports suggest no effect on cancer incidence in women but 31% ↓ in men (all cancers)
The Selenium and Vitamin E Cancer Prevention Trial (SELECT)[89]	USA	32 400 healthy men, ≥55 years	12	400 IU α-tocopherol and/or 200 μg 1-selenomethionine	Effects on prostate cancer awaited
The Prevention of Cancer by Intervention with Selenium (PRECISE) Trial[90]	Denmark, Sweden, Finland, USA, UK	40 000 healthy subjects, 60–74 years	5	100, 200 or 300 μg selenium	Effects on cancer incidence and mortality awaited
Heart Protection Study[56]	UK	20 536 men and women at high CVD risk, 40–80 years	≥5	20 mg β-carotene, 600 mg α-tocopherol and 250 mg vitamin C	No effect on cancer incidence or mortality
Vitamin A and Cancer Prevention II[46]	Australia	1204 former asbestos workers, 40–89 years	5	30 mg β-carotene or 25 000 IU retinol (no placebo group)	No effect of β-carotene on cancer mortality

Table 2 *Continued*

Study	Country	Study population	Duration of treatment (years)	Daily dose	Results
*SECONDARY PREVENTION**					
Skin Cancer Prevention Study[91]	USA	1805 men and women with recent non-melanoma skin cancer, 40–89 years	5	50 mg β-carotene	No effect on occurrence of new non-melanoma skin cancers
Nutritional Prevention of Cancer Study[92,93]	USA	1312 men and women with history of basal- or squamous-cell carcinoma, 18–80 years	4.5	200 μg selenium	No effect on incidence of skin cancer 50% ↓ in cancer mortality 37% ↓ in cancer incidence 63% ↓ in prostate cancer 58% ↓ in colorectal cancer 46% ↓ in lung cancer

CVD – cardiovascular disease.
* Secondary prevention is defined as including patients with documented cancer including non-melanoma skin cancer (although some of the primary prevention trials did not exclude those with non-melanoma skin cancer at baseline).

on the occurrence of solar keratoses (a strong determinant of squamous-cell carcinoma)[97]. A 5-year trial of 1805 men and women with recent non-melanoma skin cancer also showed that supplementation with 50 mg β-carotene provided no protection against either type of skin cancer[91], although this may have been because these cancers have a latency period of 12 years.

Together, these intervention studies have suggested that β-carotene supplements offer no protection against cancer and, amongst smokers, may actually increase the risk. Investigators have sought to explain these findings by proposing that under certain conditions β-carotene can have a pro-oxidant activity and act as a tumour promoter; high concentrations of antioxidants may shield DNA from oxidative damage, but could also protect 'initiated' cells from apoptosis and favour their clonal expression in tumour promotion[98]. Handelman *et al.* have suggested that β-carotene may be susceptible to oxidative damage from gases in cigarette smoke that may lead to the formation of harmful by-products[94,99].

To date, there have been no published randomised controlled trials of vitamin C alone in primary prevention. However, data from the small number of trials investigating vitamin C in combination with other nutrients has not provided any support for a role for high-dose vitamin C supplementation in cancer prevention. The Linxian trial found no significant effect of supplementing a population of Chinese men and women with 120 mg vitamin C and 30 μg molybdenum daily for 5 years on the risk of cancer of the oesophagus or stomach[39,100]. The Polyp Prevention Study found no evidence that either β-carotene or a combination of vitamin E and C (1000 mg) decreased the incidence of subsequent colorectal adenomas among 864 patients with previous adenoma[101], and the Heart Protection Study also found no beneficial effects of supplementation with these three vitamins on cancer mortality[56]. However, Carr and Frei have suggested that the lack of any protective effect might be because dietary intake of vitamin C was already sufficient for tissue saturation, and highlight the need for further studies in people with low vitamin C intakes[102].

There is also limited evidence from intervention trials of any benefit from vitamin E in cancer prevention. The ATBC trial showed no significant effect of daily α-tocopherol supplementation (50 mg) on risk of lung[40], pancreatic[94], colorectal[84] or urinary tract[85] cancers amongst heavy smokers. However, in a *post hoc* subgroup analysis a 34% reduction in the risk of prostate cancer (RR 0.68, 95% CI 0.53–0.78) was seen in men who received this supplement[103,104] and this reduction in risk was not demonstrated at post-intervention follow-up (6 years)[95]. Whilst these results are interesting, prostate cancer was not a primary endpoint of this study. No other studies have yet supported a preventative effect of vitamin E for prostate cancer. The Heart Protection Study found no effect of vitamin E in combination with vitamin C and β-carotene

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on cancer incidence or mortality[56]. Two smaller, short-term intervention studies found no effect of α-tocopherol supplementation on mammary dysplasia[105] or benign breast disease[106]. Several trials have also been unable to demonstrate a protective effect of vitamin E supplementation on the risk or recurrence of colorectal adenomatous polyps[98,107,108].

There is some evidence to support a protective effect of selenium supplementation on liver cancer in high-risk groups. The provision of selenium-fortified salt to a town in Qidong in China, where the inhabitants had high rates of primary liver cancer, reduced the incidence of this cancer by 35%, compared with towns that did not receive this intervention[109,110]. Intervention trials have also demonstrated the incidence of liver cancer to be significantly reduced in subjects with hepatitis B[109,111], and amongst members of families with a history of liver cancer[109,111], receiving a daily supplement of 200 μg of selenium for 4 years and 2 years, respectively.

The main intervention trial conducted to date that supports a protective role of high selenium intake against cancer is a study of 1312 patients (mostly men) in the USA, with a previous history of skin cancer, supplemented either with placebo or 200 μg selenium per day. After 4.5 years, significant reductions in the risk of total cancer incidence (37%) and mortality (50%) were observed[92]. Whilst selenium was not found to have a protective effect against recurrent skin cancer, the selenium-treated group had substantial reductions in the incidence of lung, colorectal and prostate cancers, of 46%, 58% and 63%, respectively. Further analysis showed the protective effect on prostate cancer to be confined to those with lower baseline prostate-specific antigen and plasma selenium levels[112]. Although these data need confirmation, they suggest that adequate selenium intake might be important for cancer prevention.

A large European intervention trial, Prevention of Cancer by Intervention with Selenium (PRECISE), is being set up, which will be a 5-year study of the effect of selenium supplementation at different doses on the incidence of cancer in a normal healthy population[90]. Two pilot studies are currently underway, in the UK and in Denmark. The Selenium and Vitamin E Cancer Prevention Trial (SELECT) in the USA will investigate the link between supplementation with selenium and vitamin E on the risk of prostate cancer. The SU.VI.MAX study has involved 7.5 years of supplementation with lower doses of a combination of antioxidant vitamins (120 mg vitamin C, 30 mg vitamin E, 6 mg β-carotene) and minerals (100 μg selenium, 20 mg zinc) than used in previous studies. Early reports suggest that this regime has led to a 31% and 37% reduction in cancer incidence and total mortality, respectively, compared with those receiving the placebo amongst men, but no benefit has been found amongst women (5th Congrès National de la Société Française d'Athérosclérose et de l'Arcol et de la Journée Nationale, June 2003). This inconsistent finding has been suggested to reflect higher dietary intakes of these nutrients amongst the women compared with the men in the study, but publication of these results is still awaited.

Antioxidants and COPD

The generation of oxygen free radicals by activated inflammatory cells produces many of the pathophysiological changes associated with COPD. Common examples of COPD are asthma and bronchitis, each of which affects large numbers of children and adults. Antioxidant nutrients have, therefore, been suggested to play a role in the prevention and treatment of these conditions. A number of studies have demonstrated a beneficial effect of fruit and vegetable intake on lung function[6,7,113]. For example, regular consumption of fresh fruit rich in vitamin C (citrus fruits and kiwi) has been found to have a beneficial effect on reducing wheezing, nocturnal cough and chronic cough in children[113]. Vitamin C is the major antioxidant present in the extracellular fluid lining the lung, and intake in the general population has been inversely correlated with the incidence of asthma[114], bronchitis and wheezing[115] and with pulmonary problems[116]. High-dose supplementation (1–2 g daily) has been shown to improve symptoms of asthma in adults[114] and protect against airway responsiveness to viral infections, allergens and irritants[117]. However, the beneficial effect of vitamin C has been attributed to the antihistaminic action of the vitamin rather than any antioxidant effect[69].

Other dietary antioxidants have also been positively associated with lung function. For example, decreased serum concentrations of α-tocopherol, β-carotene and vitamin C have been demonstrated in children with asthma, even during asymptomatic periods[118]. NHANES III, based on a large sample of 16 693 subjects, showed higher levels of several dietary antioxidant nutrients to be associated with better lung function in a healthy population. The combined effect on lung function of vitamin C, vitamin E, selenium and β-carotene (measured in diet and serum) was higher than for any vitamin studied alone[119]. In a study of 158 children with moderate to severe asthma, Romeiu *et al.* showed daily supplementation with vitamin E (50 mg) and vitamin C (250 mg) to lead to some improvement in lung function following ozone exposure[120]. However, the much larger ATBC trial found no benefit from supplementation with α-tocopherol (50 mg daily) and β-carotene (20 mg daily) on symptoms of COPD, despite those with high dietary intakes and blood levels of these vitamins at baseline having a lower prevalence of chronic bronchitis and dyspnea[121].

A small trial has investigated the effects of selenium supplementation in asthmatics. Those receiving the

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supplements experienced a significant increase in glutathione peroxidase levels and reported improvement in their asthma symptoms[122]. However, this improvement could not be validated by significant changes in the separate clinical parameters of lung function and airway hyper-responsiveness.

Antioxidants, macular degeneration and cataracts

The eye is at particular risk of oxidative damage due to high oxygen concentrations, large amounts of oxidisable fatty acids in the retina and exposure to ultraviolet rays. In Western countries, AMD is the leading cause of blindness among older people. Cataracts are also widespread among the elderly and occur when the lens is unable to function properly due to the formation of opacities within it. These develop when proteins in the eye are damaged by photo-oxidation; these damaged proteins build up, clump and precipitate. It has been proposed that antioxidants may prevent cellular damage in the eye by reacting with free radicals produced during the process of light absorption[123].

The carotenoids lutein and zeaxanthin are believed to be particularly important in preventing ocular damage because they are found in the pigment of the macular region of the normal retina, where they absorb blue light and protect against short-wavelength damage to the retina. A recent intervention study has shown that these carotenoids can be increased significantly by dietary supplementation[124]. Smokers who have lower plasma levels of carotenoids also have a lower macular pigment (lutein and zeaxanthin) density[125] and an increased risk of developing AMD[123]. Observational studies have examined the association between intake/plasma concentrations of antioxidant vitamins and AMD but the findings have been inconclusive and sometimes contradictory[126,127]. The results of the limited number of intervention trials have also been variable. A study in the USA investigating the effects of combined antioxidant vitamins A, C and E (with and without zinc) showed some protective effect on the progression of moderately advanced AMD[128]. However, a recent intervention trial was unable to demonstrate any alteration in the incidence or progression of AMD following 4 years of daily supplementation with 500 mg vitamin E[129]. Recent Cochrane reviews have concluded that there is no evidence from randomised trials that healthy people should take antioxidant vitamin supplements to prevent the onset of AMD. However, on the basis of the US trial, an antioxidant and mineral supplement containing vitamin E, vitamin C, β-carotene and zinc may delay the progression of the disease in people with moderate to severe AMD[130].

Many studies have demonstrated the risk of cataracts to be inversely proportional to the serum level of antioxidants[131–142]. A recent prospective study of dietary intake found that increased consumption of lutein and zeaxanthin reduces the risk of developing cataracts severe enough to require extraction[5]. The Beaver Dam Eye Study[134] did not show any strong link between five measured carotenoids and risk of cataracts but did show an inverse association with serum tocopherols[131]. In a cohort of women aged 45–67 years, high dietary carotenoid intake was found to be associated with lower risk for cataract extraction[135] and in a cohort of US male health professionals, a significantly lower risk of cataracts was found with higher intakes of β-carotene-rich foods[5]. Amongst a large group of American nurses, intake of vitamin C (but not of other antioxidants) was associated with a 69% reduction in the prevalence of lens opacities following adjustment for other nutrients[133].

Whilst the findings from these prospective studies have been encouraging, the results of intervention trials have been inconclusive. The Roche European American Cataract Trial (REACT), a randomised trial providing a combined daily supplement of β-carotene, vitamin C and vitamin E amongst adults with early signs of age-related cataract, showed a small deceleration in the progression of cataract after 3 years[136]. However, in the ATBC trial, men who were supplemented for 5–8 years with 50 mg of vitamin E or 20 mg of β-carotene or both showed no reduction in the prevalence of cataracts[137]. Moreover, a randomised, double-blind, placebo-controlled trial of over 22 000 male physicians aged 40–84 years showed no benefit from 12 years of supplementation with alternate daily β-carotene (50 mg) on the incidence of age-related cataract[138]. In fact, current smokers at the beginning of the study who received the supplement experienced an increased risk of cataract compared with the placebo group (RR 0.74, 95% CI 0.57–0.95).

Limitations of the cancer and CVD trials to date

Several reasons have been given to explain why the findings of the observational studies have differed from those of the large randomised trials. Clearly, non-randomised studies are unable to exclude the possibility that antioxidants are simply acting as a surrogate measure of a healthy diet or lifestyle. However, while intervention studies provide a more rigorous source of evidence than observational studies, they are not without weaknesses from a nutritional perspective.

There has been discussion about the nature of the supplements used in the trials. First, most have used synthetic forms that may have different biological activity or potency from the natural forms of these vitamins (although trials using the natural forms have not suggested different clinical effects from those supplementing with synthetic forms[51,53,139]). Second, concerns have been expressed about whether the correct carotenoid has been used (i.e. β-carotene vs. other carotenoids such as lycopene and lutein). However, a decline in blood

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concentrations of other carotenoids as a result of supplementation with β-carotene has not been observed in the supplementation studies[140]. Similarly, all of the trials investigating the effect of vitamin E supplementation have used α-tocopherol, the major form of vitamin E in human tissues, but this may lead to a decrease in plasma levels of γ-tocopherol[141]. This is the most prevalent form of vitamin E in plant seeds and products derived therefrom, and contributes to the body's antioxidant defences[142]. Finally, single supplements may also interfere with the uptake, transport, distribution and metabolism of other antioxidant nutrients.

Most trials have tested the effect of high doses of one or two antioxidants, while the accumulation of mechanistic and epidemiological data suggests that antioxidants act not only individually but also co-operatively, and in some cases synergistically. An optimal effect would, therefore, be expected with a combination of nutrients at levels similar to those contained in the diet (corresponding to higher levels of intake associated with reduced risk in the observational studies)[143]. However, the effects of combinations of antioxidant vitamins or multiple antioxidants remain unclear. A small randomised trial in 160 patients with coronary disease, using a combination of antioxidant nutrients (800 IU α-tocopherol, 1000 mg vitamin C, 25 mg β-carotene and 100 μg selenium, twice daily) for 3 years, showed no benefit for secondary prevention of vascular disease[139]. Similarly, neither the Heart Protection Study[56] nor early reports from SU.VI.MAX support any effect of a cocktail of antioxidant nutrients on CVD risk, but the latter study has found a reduction in cancer risk amongst men (but not women) following supplementation with a number of antioxidant vitamins and minerals. Trials have not yet investigated other potentially beneficial nutrients in antioxidant-rich foods such as flavonoids and lycopenes.

Most of the intervention trials published to date (except the PHS) had durations of treatment and follow-up lasting only around 4–6 years. The conversion of an initiated cell to a pre-malignant or fully malignant cell is a lengthy process and can take decades. Cancer is, therefore, a disease that occurs over a long period of time, and the trials may have been too short to demonstrate any benefit. Steinberg has also hypothesised that, unlike agents that lower cholesterol or blood pressure, antioxidants may have to be used for more than 5 years to have demonstrable benefit on CVD, since the primary mechanism of these agents may be in the prevention of new lesions[144].

Many of the supplementation trials have not been undertaken on normal 'healthy' individuals but on those with pre-existing oxidative stress, either through smoking or pre-existing disease, amongst whom increasing antioxidant intake may not have been able to repair the oxidative damage process sufficiently to affect cancer or CVD risk. It is also possible that some unknown genetic factors (interacting with nutrition) may explain some of the lack of effect in intervention studies. A greater understanding of the impact of factors such as genotype, age and ill health on the interactions between antioxidants and reactive oxygen species would be of help in designing future trials.

The SU.VI.MAX study has taken account of many of these issues in its design and is testing the efficacy of supplementation amongst healthy subjects over an 8-year period with a cocktail of antioxidants at doses achievable by diet. This illustrates the type of nutritional approach that may be needed in the future. However, even if future trials do demonstrate a reduction in CVD or cancer risk with antioxidant supplementation, this cannot be attributed definitively to the antioxidant effect of these nutrients, as other biological functions may also play a role. For example, as well as retarding oxidation of LDL cholesterol, vitamin E may help to protect against CVD via its action on platelet aggregation and adhesion or by inhibition of the proliferation of smooth muscle cells. Furthermore, while vitamin C, vitamin E and selenium have been shown to decrease the concentration of some of the biomarkers associated with oxidative stress, the relationship between these biomarkers and chronic disease remains to be elucidated.

The intervention studies highlight the lack of information on the long-term safety of sustained intakes of moderate to high doses of micronutrient supplements. In particular, the finding of an increased incidence of lung cancer in people at high risk taking β-carotene supplements raises the possibility that a change in the usual balance of carotenoids in the diet (for instance, by high-dose purified supplements) might lead to potentially adverse perturbations in their absorption, metabolism or function. Such findings caution against the widespread use of moderate- to high-dose micronutrient supplements, which cannot be assumed to be without adverse effects[2].

Conclusion

Although there is a substantial body of evidence that a diet rich in plant foods (particularly fruit and vegetables) conveys health benefits, as do high plasma levels of several antioxidant nutrients found in these foods, a causal link between lack of antioxidants and disease occurrence or between antioxidant administration and disease prevention remains to be established. There is a lack of understanding of the mechanisms underpinning the apparent protective effect of plant foods and, as yet, no clear picture of which components are effective, and hence no way of predicting whether all or just some plant foods are important in this respect. Further evidence is required regarding the efficacy, safety and appropriate dosage of antioxidants in relation to chronic disease. The most prudent public health advice continues to be to increase consumption of plant foods.

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