

An evidence-based nutrition
statement from the National Heart
Foundation of New Zealand's
Nutrition Advisory Committee

antioxidant supplements

Author:

D Roberts BPhEd.BSc.PGDipDiet.NZRD
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contributors

committee members

Professor J Mann PhD, MD, FRACP, FRSNZ. Professor of Human Nutrition and Medicine, University of Otago, Dunedin, New Zealand.

Dr A Chisholm DipHSc, MCApSc, PhD, NZRD. Research Dietitian, Department of Human Nutrition, University of Otago, Dunedin, New Zealand.

Associate Professor L Eyres PhD, MBA. Technical and Developments General Manager, New Zealand Dairy Foods, Auckland, New Zealand.

M McKerchar DipSc, PGDipSc (Com Nutr), NZRD. Maori Health Promoter, Southern Public Health Services, Invercargill, New Zealand.

J Reid BSc (Hons), PGDipDiet, MPH, NZRD. Nutrition Advisor, Ministry of Health, Wellington, New Zealand.

D Roberts BPhEd, BSc, PGDipDiet, NZRD. National Dietitian, the National Heart Foundation of New Zealand, Auckland, New Zealand.

Associate Professor B Swinburn MB ChB, FRACP, MD. Medical Director, the National Heart Foundation of New Zealand, Auckland, New Zealand.

A Tuffin BA, DipTchg. Senior Lecturer Health Education, College of Education, Massey University, Palmerston North, New Zealand.

L Young BHSc, PGDipSc (Com Nutr), NZRD. Food Industry Manager, the National Heart Foundation of New Zealand, Auckland, Zealand.

other contributors

J Bremer DipHSc, NZRD. Consulting Dietitian, Christchurch, New Zealand.

S Mackay BCapSc, PGDipPH, MSc. Nutritionist, Nelson, New Zealand.

M Seddon MB ChB, MPH. Public Health Registrar, the National Heart Foundation of New Zealand, Auckland, New Zealand.

reviewing organisations

The Ministry of Health, Wellington, New Zealand.

The Nutrition and Metabolism Advisory Committee of the National Heart Foundation of Australia, Canberra, Australia.

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section summary

Antioxidants occur naturally in the food supply, for example, vitamin E, β -carotene and vitamin C. These antioxidants may reduce the likelihood of the cholesterol in the blood being deposited in the artery wall and forming atherosclerotic plaques.

For the general population

A high consumption of fruits and vegetables, wholegrain breads and cereals, and suitable vegetable oils will ensure an adequate intake of vitamins and minerals.

For those at high risk of cardiovascular disease

As for the general population.

There is insufficient evidence to recommend dietary supplements of antioxidant vitamins, minerals or trace elements for the treatment or prevention of heart and blood vessel disease.

Explanatory notes

- The dietary antioxidants examined in this report have been limited to those for which there is research available, including vitamin E, β -carotene, vitamin C, flavonoids and selenium.
- Higher intakes of these antioxidants, sometimes at levels achievable only through supplementation, have been associated with lower rates of CHD in observational studies, including descriptive, case-control, and cohort studies (Level of evidence C). In some cases, this may apply only to specific groups within a region or population, for example smokers, those with low baseline blood antioxidant levels, and only when higher intakes are sustained for longer than two years.
- Supplemental vitamin E (α -tocopherol) may reduce the susceptibility of LDL-C to oxidation. However, in two large-scale RCT's (secondary prevention) vitamin E supplements had no clear beneficial effect on subsequent CVD events or mortality rates (Level of evidence A).
- The absence of demonstrated efficacy and safety in RCT's precludes the establishment of a firm recommendation on vitamin E supplements for the prevention or treatment of CVD.
- Supplemental β -carotene, 20-30 mg.d⁻¹, taken singly or combined with other antioxidants, may increase the risk of MI and total mortality (Level of evidence A).
- Supplemental vitamin C has not proven effective in reducing total morbidity or mortality from cerebrovascular disease (Level of evidence A). Further RCT's are warranted to determine whether high-dose supplementation with vitamin C has any impact on CHD end-points.
- The observational and clinical trials of flavonoids have not clearly demonstrated a benefit on cardiovascular end-points, such as CHD mortality (Level of evidence B). Where benefit has been demonstrated, an additive effect with other vitamins cannot be excluded.
- Tomatoes or tomato-based products such as tomato juice, tomato sauce, canned tomatoes and spaghetti can significantly improve lycopene concentrations in the blood when incorporated into the diet of healthy individuals (Level of evidence A). Further work is required to determine how this may translate into improved CVD outcomes.
- Further RCT's are needed utilising selenium before any recommendation can be made about its role in improving CVD outcomes.

background paper

Examination of the evidence

Introduction

Oxidative injury is suggested to be a common disease mechanism with disease end-points differing according to the identity and tissue location of the injured substrate(s). These injuries appear to be involved in many of the important causes of morbidity and mortality in New Zealand, especially cancer and CVD, but have also been implicated in such diverse conditions as stroke, arthritis, cataract formation, Parkinson's disease, and drug toxicity^{1,2,3}.

The body has many systems in place to address these oxidative injuries, including enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, and non-essential free radical scavengers such as glutathione, albumin and other proteins, uric acid, and ubiquinol-10. There are also the antioxidant vitamins, vitamin E (α -tocopherol), vitamin C (ascorbic acid) as well as the carotenoids (for example β -carotene, which is also a potential vitamin A precursor, and lycopene). A number of other dietary factors are proposed to act as antioxidants and have been suggested to protect against degenerative disease. Among these are trace elements, including copper and manganese, phytochemicals - specifically flavonoids and the mineral zinc, some of which serve as cofactors for enzymes with antioxidant activity⁴. Little information is available on the preventive effects of trace elements or minerals in human populations, therefore, the dietary antioxidants examined in this report will be limited to vitamin E, β -carotene and vitamin C for which there is extensive research. Selenium, which is essential for the important antioxidant enzyme glutathione peroxidase, and topical antioxidants, flavonoids and lycopene are also reviewed.

Observational studies

Vitamin E

Vitamin E is a generic term used to describe a group of at least eight compounds that exhibit the biological activity of α -tocopherol. The group comprises α -, β -, γ -, and δ -tocopherol, and α -, β -, γ -, and δ -tocotrienol. In the WHO/MONICA Project, lipid levels of vitamin E were strongly and inversely correlated with cross-cultural CHD mortality in 16 European populations, each of whom contributed approximately 100 healthy men, aged 40-49 years⁵.

Data from the Nurses Health Study reported that about 13% of women regularly used vitamin E supplements and, although women taking vitamin E more commonly took vitamin C and β -carotene, the effect of vitamin E was independent of these antioxidants⁶. After adjustment for known confounding factors, these women had a significant relative risk reduction of 0.69 (95% CI 0.49 to 0.97) for non-fatal MI and death from CVD compared to women who did not use supplements. However, only use of vitamin E beyond two years was associated with significant reductions in the risk for CVD. The absolute risk reduction was 3.4% per 10,000 years of follow-up (8.5 compared with 5.2 per 10,000 years of follow-up). Reduced risk was only seen with vitamin E supplementation (at least 100 IU.d^{-1†}) and not with multivitamin use (approximately 30 IU.d⁻¹).

* An atom or molecule that has one or more unpaired electron(s). This electron imbalance makes free radicals unstable and highly reactive.

† 1 IU of vitamin E is equivalent to 1 mg of synthetic α -tocopherol.

The authors further analysed the effect of vitamin E supplementation on other cardiovascular outcomes and overall mortality. For none of the categories, including overall mortality, was the association as pronounced as for major coronary disease [Table 1](#)

Similar results were found in the Health Professionals Follow-up Study⁷. In a subset of 39,910 healthy men free from CHD, those in the upper quintile of vitamin E intake (median intake 419 IU.d⁻¹) had a significant reduction in risk for non-fatal MI, death from CHD, or coronary revascularisation after adjustment for known confounders, compared to those in the lower quintile (median intake 6 IU.d⁻¹). The absolute risk reduction was 6.0 per 10,000 years of follow-up (50.9 compared with 44.9 per 10,000 years of follow-up) among persons using vitamin E supplements. Like the Nurses Health Study, use of vitamin E supplements for less than two years was not associated with a reduced risk for cardiovascular events (relative risk reduction, 0.95; $p>0.05$).

In a Finnish cohort study of 2,748 men and 2,385 women, with a 14-year follow-up, vitamin E intake tended to be related to a reduced risk for CHD mortality both among women and men⁸. It cannot be excluded that the associations observed between supplemental vitamin E intake and CHD in this and other observational studies were due to confounding caused by unsatisfactory control of various dietary and lifestyle variables closely correlated with the intake of these vitamins. For example, the Finnish study failed to adjust for vitamin C and β -carotene, nutrients proposed to have known antioxidant effects.

If such a dietary marker exists, Kushi et al failed to identify it in their study of healthy postmenopausal women⁹. The intake of vitamin E from food but not from supplements was inversely associated with mortality from CHD. Investigating whether vitamin E consumed in food was a marker for other dietary factors, the authors adjusted for carotenoids, folic acid, dietary fibre, linolenic acid, linoleic acid, total PUFAs, meat, and other foods and food groups. Adjusting for these factors did not substantially alter their findings. That the association was only found with food sources of vitamin E and not supplemental vitamin E is not in agreement with previously mentioned studies^{5,6,8}. The richest sources of vitamin E in the US diet are vegetable oils and products made from them. Meats, fish, animal fats and most fruit have little vitamin E whereas green leafy vegetables have appreciable amounts¹⁰. New important dietary sources of vegetable oil such as sunflower seed oil are high in α -tocopherol whereas corn, soybean and cottonseed oil contain other isomers ([Table 2](#)). In fact γ -tocopherol may be the predominant form of vitamin E in the diet, although it has much lower vitamin E activity¹¹.

Table 1. Relative risks for cardiovascular outcomes, according to the use of multivitamins and vitamin E supplements, after adjustment for age and coronary risk factors*.

Outcome	No. of Cases	Vitamin supplements relative risks (95% CI)	Multivitamins relative risks (95% CI)
Major coronary disease	440	0.54 (0.36 to 0.82)	0.88 (0.70 to 1.09)
Cardiovascular mortality	195	0.58 (0.30 to 1.12)	0.77 (0.58 to 1.16)
Ischaemic stroke	154	0.71 (0.39 to 1.31)	0.86 (0.59 to 1.24)
Coronary-artery surgery [†]	366	0.73 (0.48 to 1.09)	0.76 (0.59 to 0.97)
Overall mortality	974	0.87 (0.69 to 1.10)	1.10 (0.94 to 1.27)

*The risk factors included in the adjustment include age, BMI, smoking, menopausal state, postmenopausal use, exercise, regular use of aspirin, hypertension, high cholesterol, diabetes, and total energy intake.

[†]Includes coronary-artery bypass grafting and PCTA.

Table 2. Tocopherol (T)/Tocotrienol (T1) content of selected oils (mg per kilogram)

Oil	α T	α T1	β T	β T1	γ T	γ T1	δ T	δ T1	Total
Soybean	110	-	10	-	1,280	-	465	-	1,865
Sunflower	860	-	25	-	20	-	2	-	907
Palm	240	280	-	5	40	365	2	55	987
Corn	145	10	20	5	725	70	120	-	1,095

The authors acknowledge that they had no information on the duration of supplement use and, as reported by other studies, an inverse association has only been found among long-term users of supplemental vitamin E (typically greater than two years). It has also been proposed that the inclusion of short and long-term users would have weakened the overall effect of supplemental vitamin E intake if such an effect were limited only to long-term users. In addition, relatively few women in this study consumed high doses of vitamin E, compromising the study's ability to detect associations with mortality from CHD.

β -carotene

In the WHO/MONICA Project, blood concentrations of β -carotene were not significantly correlated with CHD⁵. In the Scottish Heart Health Trial, men in the highest quintile of β -carotene intake had a significantly lower risk of having unrecognised CHD than those in the lowest quintile of intake¹². As with vitamin E, ingestion of β -carotene did not reduce the risk of CHD in women. In the Nurses Health Study, women in the highest quintile of β -carotene intake had a 22% reduction in risk of CHD compared with those in the lowest quintile¹³.

A similar reduction was found in men who ingested the greatest amount of β -carotene (mean intake 19,034 IU.d⁻¹) compared to those who consumed the least (mean intake 3,969 IU.d⁻¹) in the Health Professionals Follow-up Study⁷. In this study, benefit was largely confined to current smokers, with no benefit seen in non-smokers and this finding is corroborated by several other studies that have reported an inverse association between intake of β -carotene and risk of CVD, particularly amongst smokers^{14,15,16}.

In the Finnish study of 2,748 men and 2,385 women, risk for death from CHD was not significantly reduced in men in the highest tertile of carotene intake (mean greater than 258 μ g.d⁻¹) compared with those in the lowest tertile (mean less than 147 μ g.d⁻¹) (relative risk -0.98; 95% CI -0.70 to -0.52) after adjustment for known cardiovascular risk factors⁸.

A study of male pharmaceutical employees reported that mortality from CHD was non-significantly higher in men with low baseline carotene levels (relative risk 1.53; 95% CI 1.07 to 2.20)¹⁷. Investigators of the LRC-CPPT Follow-up Study of 1,899 middle-aged men with hyperlipidaemia found that patients with blood carotenoid levels in the highest quartile (mean greater than 3.2 μ mol.L⁻¹) had a relative risk of 0.64 (95% CI 0.44 to 0.92) compared with those in the lowest quintile (mean less than 2.3 μ mol.L⁻¹)¹⁸.

A study sample of 4,802 residents (age 55-95 years) living in the district of Rotterdam, Netherlands, were followed for a period of four years to determine whether dietary β -carotene, vitamin C, and vitamin E were related to the risk of MI¹⁹. Risk of MI for the highest compared with the lowest tertile of β -carotene intake was 0.55 (95% CI 0.34 to 0.83; $p=0.013$), adjusted for known confounders. When β -carotene intakes from supplements were considered, the inverse relation with risk of MI was slightly more pronounced. Again this association was most evident in current and former smokers.

Vitamin C

Vitamin C is a descriptor for all compounds exhibiting qualitatively the biological activity of ascorbic acid. The evidence supporting an inverse association between vitamin C intake and CHD is weaker than for vitamin E and β -carotene. The Scottish Heart Health Trial and the

NHANES I Survey reported that vitamin C intake was strongly and inversely associated with CHD in men but not in women^{12,20}. Enstrom et al using data from the NHANES I Survey reported that individuals with high intakes of vitamin C exhibited significantly lower risk of death from all causes, particularly from CHD, over a 10-year follow-up period. Among men, multivariate analysis suggested a relative risk of 0.75 (95% CI 0.53 to 0.97) in individuals within the highest versus those in the lowest vitamin C intake group (50 mg.d⁻¹ dietary vitamin C plus regular supplements containing vitamin C vs. less than 50 mg.d⁻¹ dietary vitamin C). These researchers did not consider the intake of other antioxidants.

In the Nurses Health Study and the Health Professionals Follow-up Study, the use of vitamin C supplements was not significantly associated with risk of coronary events, although there was a non-significant trend towards a reduced risk in those taking supplements^{6,7}.

More recently Nyssönen et al demonstrated that men in Finland who had a vitamin C deficiency had a relative risk of acute MI of 3.5 (95% CI 1.8 to 6.7; $p=0.0002$) compared with those who were not deficient²¹. After adjustment for known risk factors for MI and dietary intake the risk reduced to 2.5 (95% CI 1.3 to 5.2; $p=0.0095$) yet still significant.

Flavonoids

Flavonoids are also purported to have antioxidant properties and are found in tea, fruits and vegetables (for example, apples, cherries, grapes, berries, onions, broccoli and legumes), and red wine. Over 4,000 different naturally-occurring flavonoids have been identified. The flavonoids group can be divided into a series of classes of compounds including flavonols, flavones, catechins, flavanones, anthocyanidins and isoflavonoids. A number of cohort studies have reported positive risk reduction from stroke, CHD, and total mortality in people that consume the greatest quantities of flavonoids.

Keli et al followed a cohort of men aged 50-69 years for a period of 15 years²². Dietary flavonoids were inversely associated with stroke incidence after adjustment for known confounders. The relative risk of the highest versus lowest quartile of flavonoids intake (greater than or equal to 28.6 mg.d⁻¹ versus less than 18.3 mg.d⁻¹) was 0.27 (95% CI 0.11 to 0.70). Approximately 70% of the daily flavonoids intake was reported to have come from black tea.

Two cohort studies have reported reductions in risk from CHD mortality ranging from 22-58% for men and 46% for women^{23,24}. From one of these studies Knekt et al also reported reductions in total mortality of 31% for women and 24% for men.

Findings are not entirely consistent with an inverse association between flavonoids and CHD mortality. Hertog et al reported that intake of SFA (73%; $p=0.0001$), flavonoid intake (8%; $p=0.01$), and the percentage of smokers per cohort (9%; $p=0.03$) explained together 90% of the variance in CHD rates across the 16 cohorts in the Seven Countries Study.

The Health Professionals Study cohort showed no associated risk reduction when comparing quintiles of flavonoid intake²⁵. After multivariate analysis the risk of non-fatal MI was 1.08 (95% CI 0.81 to 1.43) and for total CHD, 0.94 (95% CI 0.68 to 1.31). Some association was reported between total flavonoid intake and risk of death from CHD but this was limited to men who previously had CVD and was not statistically significant.

Lycopene

Like β -carotene, lycopene is a member of more than 600 naturally-occurring carotenoids that have been identified. Few studies have specifically studied lycopene itself and generally, where it has been reported, it is in the context of total carotenoid intake. The results of these trials have been reported in the section on β -carotene where risk reduction for CHD was noted at higher intakes with effect modification noted in smokers. One study reported relative risk reductions for CHD of 0.30 (95% CI 0.11 to 0.82) among current smokers, 0.60 (95% CI 0.38 to 0.94) among former smokers and 1.09 (95% CI 0.66 to 1.79) among non-smokers for total carotenoid intake⁷.

More recently a multicentre study was conducted in men with MI and matched controls from ten European countries²⁶. After adjusting for known confounders, lycopene concentration remained independently protective, with an odds ratio of 0.52 (95% CI 0.33 to 0.82; $p=0.005$) for the 10th and 90th percentiles of intake.

Selenium

This essential trace mineral allows the enzyme glutathione peroxidase to destroy hydrogen peroxide and organic hydroperoxides, providing the basis for its well-known antioxidant properties.

In a cohort of elderly Danish men, Suadicani et al reported a 70% increased risk of CHD, relative risk 1.70 (95% CI 1.14 to 2.53) in selenium deficient subjects. After multivariate adjustment, this finding remained significant, 1.55 (95% CI 1.00 to 2.39)²⁷.

Several nested case-control studies have investigated whether blood selenium predicts risk of MI^{28,29}. To-date these studies have led to indeterminate findings. Two hundred and fifty one participants in the Physicians Health Study who had infarcts and an equal number of healthy controls were matched by age, smoking status, and time from randomization²⁸. Those in the highest quintile had a relative risk of 1.27 (95% CI 0.71 to 2.29) when compared to the bottom quintile, and 1.53 (95% CI 0.61 to 3.84) after adjustment for other cardiovascular risk factors. This finding is supported by data from the Tromso Heart Study where no significant association was detected between low selenium levels and risk of MI²⁹. Kok et al actually reported low levels of selenium to be protective of MI, which persisted after multivariate adjustment of known CVD risk factors³⁰.

A much larger multicentre trial, the EURAMIC Study, assessed selenium levels in 683 non-fatal male cases with first MI and 729 matched controls³¹. After adjustment for age, centre and smoking, the odds ratio for MI in the highest quintile of selenium as compared with the lowest was 0.63 (95% CI 0.37 to 1.07; $p=0.08$). The inverse trend was stronger after adjustment for vitamin E ($p = 0.05$). Further analysis by smoking and centre suggested a stronger association in current smokers and in regions where selenium was lowest. Therefore, it may be that the risk of MI is strongest amongst smokers who live in regions of low selenium. This finding is supported by other case control studies³².

Low selenium status was a feature of New Zealanders during the 1970's and 1980's³³. This was particularly so in the South Island. More recent estimates are limited but there is some evidence that intakes have increased in the past five to ten years, at least in the South Island³⁴. The blood selenium status of Christchurch adults between 1991 and 1992 was found to be static up to approximately 1987 and then underwent a sustained increase up to 1992. This was attributed to the presence in flour, bread and other grain products of imported Australian and American wheat with higher selenium levels.

Intervention studies

Vitamin E

Angina

Angina pectoris is the mildest and often first clinical manifestation of CHD. Three RCT's were identified that examined the effect of vitamin E supplementation and angina^{35,36,37}. In the ATBC Study, 23,862 Finnish male smokers free of CHD were followed up for incidence of angina³⁵. Subjects received 50 mg.d⁻¹ vitamin E as α -tocopherol, 20 mg.d⁻¹ β -carotene, 50 mg.d⁻¹ α -tocopherol and 20 mg.d⁻¹ β -carotene combined, or placebo. A total of 1,920 new cases of angina were observed during follow-up (mean 4.7 years).

Of these, 930 occurred in the α -tocopherol supplemented subjects and 990 among the non α -tocopherol supplemented subjects, with a relative risk for incident of angina of 0.94 (95% CI 0.86

to 1.02; $p=0.15$). Compared to those who received placebo, the relative risk for the incidence of angina was 0.98 (95% CI 0.86 to 1.11; $p=0.70$) for the α -tocopherol supplemented group. The relative risk for the incidence of angina was no less in those subjects receiving both α -tocopherol and β -carotene supplements combined, 0.98 (95% CI 0.86 to 1.11; $p=0.73$). The study has received some criticism primarily because of the low vitamin E dose used. The 50 mg.d⁻¹ vitamin E dose increased blood levels by only 1.4 fold, a much lower increase than the four-fold or greater differences that observational studies suggest are needed to see a reduction in CVD^{6,7}.

Motoyama et al examined the effects of oral vitamin E administration (300 mg.d⁻¹ α -tocopherol) on endothelium-dependent vasodilation in patients with coronary spastic angina³⁷. At baseline patients with angina typically had impaired flow-dependent vasodilation and lower blood levels of α -tocopherol as compared with age-and sex-matched controls. Treatment over a period of one month resulted in restored flow-dependent dilation and ultimately reduced anginal attacks.

Presenting further analysis from the ATBC Study, Rapola et al randomized 1,795 male smokers with angina to earlier reported interventions^{35,36}. Measuring recurrence of angina there were 2,513 reported cases during the follow-up period (median 4 years). Odds ratios for recurrence as compared to placebo were 1.06 (95% CI 0.85 to 1.33) for α -tocopherol and for combination treatment, 1.02 (95% CI 0.82 to 1.27). There were no differences in progression to severe angina between treatment and placebo.

Myocardial infarction

Six RCT's addressed issues surrounding vitamin E supplementation and MI^{38,39,40,41,42,43}. Three of these studies investigated whether vitamin E supplementation would reduce the injurious effects of free radical production in acute MI^{38,39,40}. Compared to controls, all patients receiving treatment, approximately 400-600 mg per day vitamin E either alone or in combination with other antioxidants, had reduced levels of oxygen free radicals and lipid peroxides. These were small trials (mean $n = 77$) and lasted no longer than 28 days.

Stephens et al in a large scale RCT (CHAOS) of over 2,000 patients with coronary atherosclerosis tested whether treatment with a high dose of α -tocopherol would reduce risk of MI and cardiovascular death⁴¹. One thousand and thirty five patients were assigned α -tocopherol supplements containing either 800 $\mu\text{g.d}^{-1}$ or 400 $\mu\text{g.d}^{-1}$ and followed for a median period of 510 days. Alpha-tocopherol treatment significantly reduced the risk of cardiovascular death and non-fatal MI [41 vs. 64 events, relative risk 0.53 (95% CI 0.34 to 0.83; $p=0.005$)]. The beneficial effects were due to a significant reduction in the risk of non-fatal MI [14 vs. 41 events, relative risk 0.23 (95% CI 0.11 to 0.47; $p=0.005$)]. This finding is corroborated by data from the ATBC Study where the relative risk for α -tocopherol versus placebo was 0.78 (95% CI 0.56 to 1.11) for non-fatal MI³⁶.

In both the CHAOS and the ATBC Studies, there was a reported interaction between α -tocopherol and cholesterol. In the highest cholesterol tertile, α -tocopherol seemed to protect against non-fatal MI, whereas at lower cholesterol concentrations there was no such effect. This finding has been reported previously^{44,45}.

While these trials provide support for high-vitamin E supplements in the secondary prevention of CHD, recommendations to consume large amounts of vitamin E must be tempered by the fact that there were more reported cardiovascular deaths among the α -tocopherol treatment group in the CHAOS Study (27 in the treatment group vs. 23 in the placebo group)⁴¹. Total mortality was also slightly but not significantly greater in the α -tocopherol group than in the placebo group [36 (3.5%) vs. 26 (2.7%); $p=0.31$].

Rapola et al again reported data from the ATBC Study where 1,862 men, who were smokers and had a history of MI, were randomized to antioxidant vitamin supplements as previously discussed^{35,42}. The median follow-up for this group was 5.3 years with an endpoint of the first coronary event after randomisation. No significant differences were found between treatment and placebo groups on major coronary events. There were significantly more deaths from fatal CHD

in the β -carotene [1.75 (95% CI 1.16 to 2.64; $p=0.007$)] and combination therapy groups [1.58 (95% CI 1.05 to 2.40; $p=0.03$)] than in placebo.

In one of the few primary prevention trials, Virtamo et al randomized 27,271 subjects from the ATBC Study with no history of MI to vitamin supplementation for a median period of 6.1 years⁴³. The end-points for this study were primary non-fatal, acute MI and death from CHD. Neither supplement, singly or in combination, affected the incidence of non-fatal MI. Vitamin E supplementation resulted in a 8% reduction in deaths due to CHD but this was non-significant.

Blood lipids

Several small RCT's with varying end-points were identified^{46,47,48,49,50,51,52}. Mensink et al investigated the effect of tocotrienols in men with mildly-elevated blood lipids⁴⁷. After four weeks receiving 35 mg.d⁻¹ tocotrienol and 20 mg.d⁻¹ α -tocopherol, blood LDL-C concentration was unchanged. Also changes in HDL-C, TGs, Lp (a), and lipid peroxide concentrations did not differ from placebo. The supplements appeared to have no favourable effect on blood lipids.

Jialal and Grundy used a combination of antioxidant supplements (800 IU.d⁻¹ α -tocopherol, ascorbate 1.0 g.d⁻¹, and β -carotene 30 mg.d⁻¹) in their RCT examining lipid peroxidation⁴⁸. Compared with placebo, all blood levels of the vitamins were increased (2.6, 4.1 and 16.3 fold respectively). At three months, combined supplementation resulted in a two-fold prolongation of lag phase and a 40% decrease in oxidation rate of LDL-C. Comparing the combination therapy to 800 IU.d⁻¹ of α -tocopherol alone, showed no significant differences between the two groups with respect to LDL-C oxidation.

Mosca et al demonstrated a reduced susceptibility of LDL-C to oxidation in 45 patients with CVD⁵⁰. The intervention group received either 400 IU.d⁻¹ vitamin E, 500 mg.d⁻¹ vitamin C, 12 mg.d⁻¹ β -carotene; or 800 IU.d⁻¹ vitamin E, 1,000 mg.d⁻¹ vitamin C and 24 mg.d⁻¹ β -carotene. Lag phase significantly increased from baseline to 12 weeks in the high-dose supplement group ($p<0.01$). A non-significant increase in lag phase in the smaller dose group was also noted during the same time period.

Simons et al administered three doses of vitamin E to a group of healthy people in an attempt to determine minimum dose required to reduce LDL-C oxidation⁵². Three doses examined were 500 IU.d⁻¹, 1,000 IU.d⁻¹ or 1,500 IU.d⁻¹. Compared to placebo, the median prolongation in time lag on 500 IU.d⁻¹ was 26%, on 1,000 IU.d⁻¹ 24% and on 1,500 IU.d⁻¹, 35%. The corresponding slowing in oxidation rates was 14%, 19%, and 25% respectively.

The results indicate a threshold effect where 500 IU.d⁻¹ appears to be able to significantly reduce the susceptibility of LDL-C to oxidation in healthy participants.

Restenosis

Three RCT's included vitamin E in their investigations on restenosis^{53,54,55}. In the largest of these trials, 317 patients were assigned probucol (500 mg.d⁻¹), multivitamins (30,000 IU.d⁻¹ β -carotene, 500 mg.d⁻¹ vitamin C, and 700 IU.d⁻¹ vitamin E), or both probucol and multivitamins, all given twice daily⁵³. Patients were treated four weeks before and six months after angioplasty. Patients also received an additional 1,000 mg.d⁻¹ of probucol, 2,000 IU.d⁻¹ of vitamin E, both probucol and vitamin E, or placebo 12 hours prior to angioplasty. Restenosis rates per segment were 20.7% in the probucol group, 28.9% in the combined treatment group, 40.3% in the multivitamin group, and 38.9% in the placebo group. While this trial does not specifically investigate vitamin E, that the multivitamins in general had no significant effect on restenosis should be eluded too. The possibility that the vitamins used in the study, including those administered 12 hours prior to angioplasty, paradoxically acted as pro-oxidants has been suggested. This might explain the tendency for probucol to have better results when given alone, than when combined with multivitamins.

DeMaio et al administered 1,200 IU.d⁻¹ α -tocopherol to patients for four months post PTCA⁵⁵. Patients receiving treatment had a 35.5% restenosis versus 47.5% restenosis in patients

receiving placebo. This difference ($p=0.06$) did not reach significance possibly because of the smaller sample size or lack of intervention prior to PTCA as documented elsewhere.

β -carotene

Many of the RCT's that examined vitamin E supplementation and CVD at the same time used β -carotene, so there is much overlap. Several additional RCT's have also been identified that have investigated similar end-points.

Angina

The largest trials have been those drawing on participants from the ATBC Study^{35,36,42,43,56}. Investigating the effect of β -carotene on angina pectoris Rapola et al reported no significant effect of 20 mg.d⁻¹ β -carotene on incidence or rate of progression to a more severe state, either singly, or in combination with α -tocopherol³⁵. In fact it was reported that male smokers were significantly more likely to develop angina if they consumed β -carotene, relative risk 1.13 (95% CI 1.00 to 1.27).

Myocardial infarction

Investigating a therapeutic role in the prevention and management of MI, trials have reported increased risk for participants using β -carotene supplements. Rapola et al reported that the risk of fatal CHD was significantly increased in the β -carotene supplemented participants, 1.75 (95% CI 1.16 to 2.64; $p=0.007$) and the combined group, 1.58 (95% CI 1.05 to 2.40; $p=0.03$)⁴². Relative risk of MI was increased across all groups but greatest in the β -carotene only group, 3.44 (95% CI 1.70 to 6.94). In participants with no history of MI, the incidence of major coronary events increased 1% (95% CI to 10% to 10%), with no effect on non-fatal MI or fatal CHD.

Overall mortality in the ATBC Study has also been reported to be 8% higher in the group supplemented with β -carotene compared to those that did not receive the vitamin (95% CI 1 to 16%)⁵⁶. This is in agreement with other large RCT's where active treatment participants (30 mg per day β -carotene and 25,000 IU.d⁻¹ retinol) in the CARET trial had a 17% higher risk of death from all causes as compared to placebo 1.17 (95% CI 1.03 to 1.33; $p=0.02$)⁵⁷. Analysis according to the cause of death showed that relative risk of death from CVD was 1.26 (95% CI 0.99 to 1.61). Mean follow-up in this study was approximately four years. Analysis of each individual vitamin was not published.

In contrast, Greenberg et al found that patients assigned β -carotene supplements (50 mg.d⁻¹) had mortality rates very similar to patients assigned placebo⁵⁸. Mortality from all causes and CVD were found to be associated with initial blood β -carotene concentration, something that had not been investigated in previous RCT's. Each was approximately 40% lower among patients in the higher two quartiles of blood β -carotene concentration (greater than or equal to 0.42 $\mu\text{mol.L}^{-1}$), relative to those in the lowest quartile (less than or equal to 0.14 $\mu\text{mol.L}^{-1}$). Multivariate analysis blunted these apparent protective effects only slightly [relative risk for total mortality 0.62 (95% CI 0.44 to 0.87; $p=0.001$) and for CVD deaths 0.57 (95% CI 0.34 to 0.95% CI; $p=0.03$)].

An examination of the β -carotene component of the Physicians Health Study also reports no significant benefit or harm from 50 mg.d⁻¹ β -carotene supplementation on alternate days⁵⁹. This study was much longer than those previously reported, with treatment and follow-up lasting 12 years.

Blood lipids

Few RCT's have investigated whether β -carotene can reduce lipid peroxidation. Of those identified, 12 to 100 mg.d⁻¹ β -carotene doses failed to reduce the lag phase to oxidation below that which placebo was able to achieve in both healthy and at risk subjects^{48,50,60,61,62}. This was often despite six to 16-fold increases in either blood or LDL-C β -carotene concentrations.

In other smaller RCT's, van Poppel et al have reported that 20 mg.d⁻¹ could not significantly alter haemostatic variables and blood lipids in a group of male smokers^{63,64}.

The equivocal findings for β -carotene may be due to the different preparations of β -carotene used in the trials. Absorption of β -carotene from supplement preparations can be higher (up to 50%) than vegetables (up to 20%). Food form is also important, where cooked, pureed or finely chopped vegetables give higher absorption than raw vegetables, largely through liberation of the carotenoids through cell disruption and hydrolysis of complexes. The presence of other dietary factors such as fibre, chlorophyll and other carotenoids may also modify absorption. In several trials examining the absorption of carotenoids from cooked or processed vegetables as part of a meal compared with a capsule of β -carotene in the same meal, much better absorption from the capsule was observed^{65,66}. Elevation of blood β -carotene from carrots was only 20-30% of that from the capsule. However, some foods increased blood concentrations of other carotenoids.

All carotenoids in blood can be effectively increased by a mixed diet rich in carotenoid-containing foods as demonstrated by a US study⁶⁷. Yeum et al reported that blood concentrations of lutein, cryptoxanthin, α -carotene, β -carotene and lycopene were all significantly increased ($p < 0.05$) on days six to 16 following a high fruits and vegetables diet⁶⁷. The magnitude of the increase was probably related to baseline carotenoid concentrations.

Vitamin C

Seven RCT's were identified that examined vitamin C and CVD^{68,69,70,71,72,73,74}. In the largest of these trials conducted in a region of China, a combination of vitamin C and molybdenum did not reduce total mortality, relative risk -1% (95% CI to 10% to 7%) or mortality from cerebrovascular disease, relative risk -4% (95% CI -24% to 12%). The 120 mg.d⁻¹ dose led to a five-fold increase in blood levels of vitamin C suggesting that lack of benefit was not because of sub-optimal dosage.

Ghosh et al investigated vitamin C effect on blood pressure in a small group of patients with systolic or essential hypertension⁷⁰. Receiving 500 mg.d⁻¹, clinically significant falls in both SBP [-10 (95% CI 0.70 to 20.0; $p=0.05$) mm Hg] and DBP [-5.9 (95% CI 0.2 to 11.5; $p=0.05$) mm Hg] were observed. However, the authors found no statistical difference between the effects of the treatment group and placebo.

Three RCT's investigated the effect vitamin C has on blood lipids with inconclusive trends^{71,72,74}. In the first trial, patients with established CHD were asked to consume 4.5 g.d⁻¹ of ascorbate acid for 12 weeks and Lp (a) measured. This dosage was well-tolerated and produced a marked elevation in blood ascorbate acid levels. No significant effect was observed on blood Lp (a). The second RCT examined nineteen, healthy smokers who received 1,000 mg.d⁻¹ ascorbate acid for a period of four weeks. Significantly increased time lag and reduced susceptibility of LDL-C to oxidation were observed in the intervention group. This finding is not corroborated by previously reported studies where combinations of antioxidants have failed to demonstrate any significant measurable effect on LDL-C oxidation, over that which vitamin E provides alone^{48,50,74}. Mulholland et al failed to show any significant effect in female smokers following a similar dose to that describe above⁷⁴.

A single trial was identified that examined vitamin C and restenosis following PTCA. A total of 119 patients with the presence of either stable or unstable angina and who had undergone PTCA were included in this trial. Patients received 500 mg.d⁻¹ ascorbic acid for a period of only four months. At four months, minimal luminal diameter was significantly larger ($p < 0.01$) in the

treatment group than placebo. Lesion-based analysis reported 39% in placebo versus 22% in treatment ($p < 0.05$).

While vitamin C is rapidly and efficiently absorbed from the diet there is uncertainty over whether vitamin C is better absorbed from citrus fruits or juices or in a supplement. Vitamin C alone appears to be slightly better absorbed than from fruit juice. However, commercial fruit juice is often processed to remove flavonoids. These compounds appear to enhance absorption, and vitamin C from a native citrus extract has been shown to be more bioavailable than vitamin C alone¹.

Flavonoids

Four RCT's examined flavonoid intake and modification of CVD risk factors, specifically the oxidation of LDL-C^{75,76,77,78}. Most studies were very small (mean $n=13$) and had relatively short treatment phases (approximately three weeks). Ishikawa reported inhibited macrophage-mediated LDL-C oxidation and increased time lag before LDL-C oxidation took place after consumption of 750 ml.d⁻¹ (approximately 5 cups) of black tea for a period of four weeks⁷⁵.

The positive effects on LDL-C oxidation have also been reported using black currant and apple juices as sources of flavonoids⁷⁶. Three doses of juice (750 ml.d⁻¹, 1,000 ml.d⁻¹ and 1,500 ml.d⁻¹) were consumed for one week. The response cannot specifically be attributed to flavonoids and indeed blood ascorbic acid was shown to increase during the treatment phase. Other studies have demonstrated that the effects of flavonoids and vitamin C on biochemical outcomes can be additive⁷⁹.

Two other RCT's have failed to find any treatment effect of flavonoids on LDL-C oxidation^{77,78}. One study using male and female smokers could demonstrate no significant effect on blood lipids following consumption of 900 ml.d⁻¹ (approximately 6 cups) of black or green tea⁷⁷. Using coffee as a control and black tea as treatment, McAnlis et al also reported no significant difference in the total blood antioxidant capacity or susceptibility of LDL-C to oxidation⁷⁸. With no increase in blood antioxidants detected, it may be that the treatment dose in these trials was not sufficiently strong enough to produce a measurable change.

Some of the interest in flavonoids has been stimulated by the finding that the phenolic compounds in red wine can inhibit in vitro oxidation of LDL-C^{80,81}. However, in a more recent RCT de Rijke et al demonstrated that the consumption of 550 ml.d⁻¹ red wine by healthy volunteers did not affect the susceptibility of LDL-C to oxidation⁸². Concentrations of measured antioxidants were unchanged after red wine consumption. These differing results may be attributed to differences in methodology so the question of the effects of flavonoids in red wine remains to be settled.

Lycopene

Several RCT's have examined lycopene, however, only one has sought to identify lycopenes role in modification of CVD risk factors. In this small trial, 19 healthy subjects were provided with instruction to increase dietary lycopene from food sources such as tomato juice and spaghetti sauce⁸³. Over a one-week period, dietary instruction had led to a two-fold increase in blood lycopene concentrations. Although there was no change in blood cholesterol levels, blood lipid peroxidation and LDL-C oxidation were significantly decreased.

Other RCT's have identified the bioavailability of lycopene from various food sources and its interaction with other nutrients, specifically β -carotene^{84,85,86}. Tomato-based products have received the most attention and have been able to significantly improve blood lycopene concentrations when incorporated into the diets of healthy subjects. Food form may also be an important determinant of blood lycopene concentrations, where some carotenoids maybe more bioavailable in juice form rather than from raw or cooked vegetables⁸⁵.

Supplementation trials have demonstrated that blood lycopene concentrations are enhanced when co-ingestion with β -carotene supplements occurs⁸⁶.

Clearly more work is required to identify how lycopene might positively modify CVD risk factors and how this might translate into a suitable recommendation for patients to follow.

Selenium

Only one RCT could be identified that examined selenium and CVD⁸⁷. In this trial, 81 patients with MI were treated with a selenium-rich yeast ($100 \mu\text{g}\cdot\text{d}^{-1}$) for a period of six months. Blood selenium concentrations rose 1.5-fold in the treatment group. During the six months of follow-up, there were fewer cardiac events compared to placebo. Further trials are needed to confirm these findings and evaluate the efficacy of selenium in the prevention and management of acute MI.

Biological mechanisms

The association between intakes of these antioxidants and reduced incidence of CVD has often been explained by the "oxidative modification hypothesis" of atherosclerosis (for full review refer to Diaz et al⁸⁸). This proposes that atherogenesis is initiated by oxidation of the lipids in LDL-C, also termed lipid peroxidation and as been much of the focus for antioxidants in RCT's.

There is difficulty, however, in linking the reduced oxidation of LDL-C to inhibition of atherosclerosis. Coronary heart disease is a dynamic process that involves not only the development of an atherosclerotic plaque but also plaque rupture, vasoconstriction, and local thrombosis, resulting in partial or total arterial obstruction. Earlier discussion also suggests that β -carotene and vitamin C may not have a significant effect on LDL-C oxidation compared to vitamin E.

As well as acting as an antioxidant to protect lipid components, vitamin E also protects membranes and lipoprotein. The chemical reactions that are involved in the neutralisation of free radicals by vitamin E have not been fully delineated. When radicals react with α -tocopherol, the tocopheryl radical is formed. This may eventually be excreted via the liver or may be regenerated (possibly by vitamin C) back to α -tocopherol. The tocopherols are also able to quench singlet oxygen, but are poor at this compared to the carotenoids¹.

The carotenoids appear to be efficient quenchers of singlet oxygen and can directly scavenge free radicals. Some variability in antioxidant activity among the carotenoids has been observed in vitro, for example, lycopene exhibits superior antioxidant capability compared with β -carotene. Carotenoids also exist in different isometric forms (*cis* and *trans* isomers) and there is some speculation that there are isomer-specific biological functions for the carotenoids¹.

In contrast to the lipid soluble antioxidant vitamins, vitamin C performs its role as a reducing agent in the blood. It may also be involved in reducing other antioxidants, such as vitamin E, to restore their function, although this has yet to be clearly established. Vitamin C effectively scavenges a wide variety of radical species and oxidants including superoxide, hydrogen peroxide, aqueous peroxy radical, hydroxyl radicals, hypochlorous acid and singlet oxygen¹.

Diaz et al propose several mechanisms aside from those related to reduced oxidation of LDL-C whereby antioxidants may have a role⁸⁸. These include preservation of endothelium-derived nitric oxide action, inhibition of leucocyte adhesion, reduction of cellular oxidative injury, and inhibition of platelet activation and smooth-muscle proliferation.

For example, endothelial cells and macrophages exposed to oxidised LDL-C rapidly become necrotic whereas, endothelial cells and macrophages loaded with α -tocopherol are resistant to the cyto-toxic effects of oxidised LDL-C. Much of the research for these mechanisms stems from animal or in vitro studies and needs to be confirmed in clinical studies on humans.

Safety of antioxidant supplements

The safety of prolonged intakes of large doses of antioxidant supplements has not yet been fully established. Certainly in the larger RCT's conducted to date (ATBC Study, CHAOS and CARET trial), adverse effects on CVD and total mortality rates have been recorded for vitamin E, β -carotene and vitamin A^{41,42,56,57}. Higher incidence of some cancers has also accompanied large doses of antioxidants in specific populations⁵⁷.

For vitamin E, the suggestion has been that at high intakes (greater than 400 IU.d⁻¹) it may act as a pro-oxidant. In addition, concerns have been raised about the effect of widespread supplementation with α -tocopherol on the other vitamin E isomers. It may be that α -tocopherol displaces γ -tocopherol which may itself have an important metabolic role despite its much lower tissue and blood levels¹.

More evidence is needed to determine the long-term safety of supplemental β -carotene. When ingested in large doses for weeks or years, the carotenoids are not known to be toxic possibly due to their reduced absorption at high doses. As reported earlier, 50 mg β -carotene on alternate days produced no benefit or harm to healthy male physicians over a period of 12 years⁵⁹. Baseline levels of carotenoids and the presence of other high-dose supplements may impact upon disease outcomes⁶⁷.

Adverse effects of high doses of vitamin C have been suggested by occasional reports. These include renal effects, effects on blood and coagulation associated with increased absorption of non-haeme iron with vitamin C, drug interactions and migraines. Osmotic diarrhoea has been identified as the most likely adverse effect in healthy individuals who consume high doses. Despite these occasional reports, the widespread use of vitamin C supplements suggests that the risk is very small although ingestion of large amounts of vitamin C cannot be recommended¹.

For selenium, the level required to cause toxicity is not known but adverse effects have been noted for intakes of approximately 5,000 $\mu\text{g}\cdot\text{d}^{-1}$ ¹³³. Selenium also plays a role with iodine in thyroid hormone metabolism and supplementation may exacerbate an existing condition¹.

Finally flavonoids have been shown to produce toxic effects when used in drug preparations. Doses of 0.5-1.5 g.d⁻¹ may cause renal failure, haemolytic anaemia, hepatitis, and a range of other symptoms^{89,90}.

conclusion

With oxidative injury purported to have a role in so many degenerative diseases it is not surprising that interest in antioxidants from both the public as well as the medical field has increased. Consistent with this are the numerous observational studies that have demonstrated lower disease risk at higher antioxidant intakes. Included amongst these are suggestions that the therapeutic effects of antioxidants can only be achieved by supplementation. A large number of RCT's have been conducted now, with inconclusive findings.

Notably missing is direct evidence from large scale RCT's of the protective effects of antioxidants in human populations that are initially free of any risk factors for CVD. Also few trials have been established to directly measure CVD end-points. Some have, and results from secondary prevention trials have shown beneficial effects of vitamin E supplements. In contrast, trials examining β -carotene supplementation have not shown any positive effect and, in some cases, have demonstrated harm, particularly among high-risk groups. More trials are needed examining other antioxidant nutrients.

Given these findings and until long-term safety can be assured, recommendations for the general population should continue to emphasise increased consumption of plant-based foods to ensure adequate vitamin and mineral intake. For those at risk or with established CVD, the evidence for vitamin E is encouraging but more work is required. Such variables as optimal dose, who is most

likely to benefit and length of treatment need to be addressed. Until these questions are answered, firm recommendations on supplementation cannot be made.

evidence tables

Evidence Table 3: Vitamin E & CHD

Key Words: Vitamin E, α -tocopherol, RCT's, CVD, MI

Reference: Stephens NG, Parsons A, Schofield PM et al. Randomized controlled trial of vitamin E in patient with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 1996; 347: 781-86⁴¹.

Study Type/Grade	Randomized Controlled Trial Grade A																																													
Outcomes	Primary: Cardiovascular death and non-fatal MI Secondary: Non-fatal MI																																													
Design	N= 2,002. Relevant risk factors: Coronary atherosclerosis, diabetes, smoking status, left ventricular impairment, and family history. Inclusion and exclusion criteria: Inclusion criteria included angiographically-proven coronary atherosclerosis. There were no exclusion criteria except prior use of vitamin supplements containing vitamin E. Power: 80% power to detect a relative risk of the combined endpoint of less than 0.75 between treatment groups. Method of randomization: Random number database to allocate treatment by blocks of two after clinical data had been entered. Intervention: Capsules of α -tocopherol 400 or 800 IU.d ⁻¹ in one daily dose. Blinding: Double-blind placebo controlled. Length of follow-up: median 510 days. Completeness of follow-up: 98%.																																													
Validity	Is the study type appropriate for the question being asked? Yes. Was the study population typical of patients with this disease? Yes. Were the treatment/control groups comparable at baseline? Small differences between active treatment and placebo groups in sex ratio, blood total cholesterol, SBP, presence of diabetes, and the proportion taking β -blockers. Was the intervention compared to placebo and/or best-accepted intervention? Yes. Was there compliance with the intervention? No difference between groups in proportion who were compliant. Was there equal intensity of observation of study and control subjects? No clinical follow-up planned as part of trial. Was the process of observation likely to effect the outcome? No. Intention to treat analysis? Yes. Did conclusions about safety take into account the limited size of the study? Yes. Is effectiveness proven? No. Summary: Valid study, well-designed.																																													
Results	Quantified results: Distribution of non-fatal MI and deaths by certified cause in each treatment group. <table border="1"> <thead> <tr> <th></th> <th>α-tocopherol Group (n = 1,035)</th> <th>Placebo Group (n = 967)</th> </tr> </thead> <tbody> <tr> <td>Non-fatal MI</td> <td>14</td> <td>41</td> </tr> <tr> <td>Cardiovascular death</td> <td></td> <td></td> </tr> <tr> <td> Fatal MI</td> <td>18</td> <td>13</td> </tr> <tr> <td> Left ventricular failure</td> <td>5</td> <td>8</td> </tr> <tr> <td> Stroke</td> <td>1</td> <td>1</td> </tr> <tr> <td> Ruptured AAA</td> <td>2</td> <td>0</td> </tr> <tr> <td> Cardiac arrhythmias</td> <td>1</td> <td>1</td> </tr> <tr> <td>Total cardiovascular events</td> <td>27</td> <td>23</td> </tr> <tr> <td>Other causes of death</td> <td></td> <td></td> </tr> <tr> <td> Pulmonary embolism</td> <td>3</td> <td>1</td> </tr> <tr> <td> Septicaemia</td> <td>2</td> <td>0</td> </tr> <tr> <td> Bowel cancer</td> <td>4</td> <td>1</td> </tr> <tr> <td> Unknown</td> <td>0</td> <td>1</td> </tr> <tr> <td>Total deaths</td> <td>36</td> <td>26</td> </tr> </tbody> </table>		α -tocopherol Group (n = 1,035)	Placebo Group (n = 967)	Non-fatal MI	14	41	Cardiovascular death			Fatal MI	18	13	Left ventricular failure	5	8	Stroke	1	1	Ruptured AAA	2	0	Cardiac arrhythmias	1	1	Total cardiovascular events	27	23	Other causes of death			Pulmonary embolism	3	1	Septicaemia	2	0	Bowel cancer	4	1	Unknown	0	1	Total deaths	36	26
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Authors' Conclusions	"Patients with angiographically – proven, symptomatic coronary atherosclerosis, α -tocopherol treatment substantially reduces the rate of non-fatal MI, with beneficial effects apparent after one year of treatment".																																													
Reviewers' Conclusions	Proven benefit on non-fatal MI however requires further investigation to explain larger number of cardiovascular deaths and total mortality following α -tocopherol supplementation at 400-800 IU.d ⁻¹ .																																													

Evidence Table 4: Vitamin E, β -carotene & CHD

Key Words: ATBC Study, α -tocopherol, β -carotene, primary CHD

Reference: Virtamo J, Rapola JM, Ripatti S et al. Effect of vitamin E and β -carotene on the incidence of primary non-fatal myocardial infarction and fatal coronary heart disease. *Arch Intern Med* 1998; 158: 668-75⁴³.

Study Type/Grade	Randomized Controlled Trial Grade A																				
Outcomes	Primary: Primary non-fatal MI Secondary: CHD mortality																				
Design	N= 27,271. Relevant risk factors: Smoking status, history of angina, history of diabetes. Inclusion and exclusion criteria: Participants were male smokers (≥ 5 cigarettes per day at entry) aged 50-69 years. Exclusion criteria were proven malignancy, severe angina pectoris, chronic renal insufficiency, cirrhosis of the liver, alcoholism, other medical problems that might limit participation, use of anticoagulants, or use of supplements vitamin E, vitamin A, or β -carotene in excess of pre-defined doses. Power: Not specified. Method of randomization: 2 x 2 factorial design. Intervention: Assigned to 1 of 4 supplementation regimens: vitamin E 50 mg.d ⁻¹ , vitamin E 50 mg.d ⁻¹ and β -carotene 20 mg.d ⁻¹ , β -carotene 20 mg.d ⁻¹ , and placebo. Blinding: Double-blind placebo-controlled. Length of follow-up: Median 6.1 years. Completeness of follow-up: Drop out rates similar in the four intervention groups ranging from 26.4% to 27.2%.																				
Validity	Is the study type appropriate for the question being asked? Yes. Was the study population typical of patients with this disease? Single gender study, specific to smokers. Were the treatment/control groups comparable at baseline? Yes. Was the intervention compared to placebo and/or best-accepted intervention? Yes. Was there compliance with the intervention? Yes. Was there equal intensity of observation of study and control subjects? Yes. Was the process of observation likely to effect the outcome? No. Intention to treat analysis? Partially abandoned when studying the effects of duration of the supplementation and self-perceived yellowing of the skin. Did conclusions about safety take into account the limited size of the study? Yes. Is effectiveness proven? No. Summary: Valid study, well-designed.																				
Results	Quantified results: Incidence (per 1,000 person-years) and relative risk (RR) of primary major coronary events among men without prior MI by supplementation in the ATBC Study. <table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th>Coronary Event</th> <th>Vitamin E</th> <th colspan="2">Vitamin E and β-Carotene</th> </tr> <tr> <th></th> <th></th> <th>β-Carotene</th> <th>β-carotene</th> </tr> </thead> <tbody> <tr> <td>All cases</td> <td>0.98 (0.87 to 1.10)</td> <td>0.97 (0.86 to 1.09)</td> <td>1.03 (0.91 to 1.16)</td> </tr> <tr> <td>Non-fatal MI</td> <td>1.04 (0.89 to 1.22)</td> <td>0.99 (0.84 to 1.16)</td> <td>1.06 (0.90 to 1.24)</td> </tr> <tr> <td>Fatal CHD</td> <td>0.90 (0.75 to 1.08)</td> <td>0.94 (0.79 to 1.13)</td> <td>0.99 (0.83 to 1.19)</td> </tr> </tbody> </table>	Coronary Event	Vitamin E	Vitamin E and β -Carotene				β -Carotene	β -carotene	All cases	0.98 (0.87 to 1.10)	0.97 (0.86 to 1.09)	1.03 (0.91 to 1.16)	Non-fatal MI	1.04 (0.89 to 1.22)	0.99 (0.84 to 1.16)	1.06 (0.90 to 1.24)	Fatal CHD	0.90 (0.75 to 1.08)	0.94 (0.79 to 1.13)	0.99 (0.83 to 1.19)
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Authors' Conclusions	"Supplementation with a small dose of vitamin E has only a marginal effect on the incidence of fatal CHD in male smokers with no history of myocardial infarction. Supplementation with β -carotene has no primary preventive effect on major coronary events".																				
Reviewers' Conclusions	Agree with authors' conclusion as reduction in CHD failed to reach statistical significance. The trial could be criticised for using such a small dose of vitamin E. Await trials using higher doses of these vitamins.																				

Evidence Table 5: β -carotene, vitamin A & CVD

Key Words: CARET, β -carotene, vitamin A, CVD and lung cancer

Reference: Omenn GS, Goodman GE, Thornquist MD et al. Effects of a combination of beta carotene and vitamin. A on lung cancer and cardiovascular disease. *N Engl J Med* 1996; 334: 1150-55⁵⁷.

Study Type/Grade	Randomised Controlled Trial Grade A																		
Outcomes	Primary: Incidence of lung cancer Secondary: Total mortality																		
Design	N= 18,314. Relevant risk factors: Smoking status, exposure to asbestos, gender, race and age. Inclusion and exclusion criteria: To be eligible, subjects had to have first been exposed to asbestos for 15 years, asbestos-related lung disease or worked in specified high-risk trade. Were required to be current smokers, or to have smoked within previous 15 years. For recruited, smoking population had to be aged 50-69 years, had at least 20 pack-year cigarette smoking, and either were currently smoking or had quit smoking within previous six years. Power: Not specified. Method of randomization: 2 x 2 factorial design. Intervention: 30 mg.d ⁻¹ β -carotene and 25,000 IU.d ⁻¹ vitamin A Blinding: Double-blind, placebo-controlled. Length of follow-up: Mean 4.0 years. Completeness of follow-up: Not specified.																		
Validity	Is the study type appropriate for the question being asked? Yes. Was the study population typical of patients with this disease? Yes. Were the treatment/control groups comparable at baseline? Yes. Was the intervention compared to placebo and/or best-accepted intervention? Yes. Was there compliance with the intervention? Yes. Among active participants, mean capsule consumption was 93%. Was there equal intensity of observation of study and control subjects? No, active participants visited a study centre once and were telephoned twice. Inactive participants were telephoned semi-annually. When results of ATBC Study published, research committee requested that blinding be ended. Was the process of observation likely to effect the outcome? No. Intention to treat analysis? Yes. Did conclusions about safety take into account the limited size of the study? Yes. Is effectiveness proven? No. Summary: Different intensity of observation and subsequent lack of blinding may have affected validity of this study.																		
Results	Quantified results: Incidence and estimated relative risk of death from all causes. <table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th rowspan="2"></th> <th colspan="2">Deaths/1,000 Person -Yr</th> <th rowspan="2">Deaths from All Causes Relative Risk</th> </tr> <tr> <th>Active Treatment</th> <th>Placebo</th> </tr> </thead> <tbody> <tr> <td>All subjects</td> <td>14.45</td> <td>11.91</td> <td>1.17 (95% CI 1.03 to 1.33; <i>p</i> = 0.02)</td> </tr> <tr> <td>Workers exposed to asbestos</td> <td>17.76</td> <td>14.30</td> <td>1.25 (95% CI 1.01 to 1.56; <i>p</i> = 0.04)</td> </tr> <tr> <td>Heavy smoker</td> <td>13.26</td> <td>10.91</td> <td>1.13 (95% CI 0.96 to 1.32; <i>p</i> = 0.14)</td> </tr> </tbody> </table>		Deaths/1,000 Person -Yr		Deaths from All Causes Relative Risk	Active Treatment	Placebo	All subjects	14.45	11.91	1.17 (95% CI 1.03 to 1.33; <i>p</i> = 0.02)	Workers exposed to asbestos	17.76	14.30	1.25 (95% CI 1.01 to 1.56; <i>p</i> = 0.04)	Heavy smoker	13.26	10.91	1.13 (95% CI 0.96 to 1.32; <i>p</i> = 0.14)
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Reviewers' Conclusions	Agree that in those at risk, combined supplementation with β -carotene and vitamin A in the amounts specified may contribute to increased mortality and should not be recommended for this group.																		

abbreviations

AHA	American Heart Association
ATBC	Alpha Tocopherol, Beta Carotene Cancer Prevention Study
BMI	Body Mass Index
CARE	Cholesterol and Recurrent Events Trial
CARET	Beta Carotene and Retinol Efficacy Trial
CCPT	Chicago Coronary Prevention Trial
CHAOS	Cambridge Heart Antioxidant Study
CHO	Carbohydrate
CHD	Coronary Heart Disease
CI	Confidence Interval
CVD	Cardiovascular Disease
DART	Diet and Reinfarction Trial
DASH	Dietary Approaches to Stop Hypertension
DBP	Diastolic Blood Pressure
DHA	Docosahexenoic Acid
DNSBA	Dietary and Nutrition Survey of British Adults
EPA	Eicosapentenoic Acid
EURAMIC	European Antioxidant Myocardial Infarction and Breast Cancer
GI	Glycaemic Index
HDL-C	High Density Lipoprotein Cholesterol
INTERSALT	International Study of SALT
LED	Low Energy Diet
LDL-C	Low Density Lipoprotein Cholesterol
LNA	Alpha Linolenic Acid
Lp (a)	Lipoprotein (a)
LRC-CPPT	Lipid Research Clinic Coronary Primary Prevention Trial
MEDLINE	Medical Literature Analysis and Retrieval System Online
MeSH	Medical Subject Heading
MI	Myocardial Infarction
MONICA	Monitoring of Trends and Determinants in Cardiovascular Disease
MRFIT	Multiple Risk Factor Intervention Trial
MUFA	Monounsaturated Fat
NAS	Normative Aging Study
NCEP	National Cholesterol Education Program
NHANES	National Health and Nutrition Examination Survey

NHLBI	National Heart, Lung and Blood Institute
NSP	Non-Starch Polysaccharide
NZ	New Zealand
NZDA	New Zealand Dietetic Association
OPPT	Oslo Primary Prevention Trial
PTCA	Percutaneous Transluminal Coronary Angioplasty
PUFA	Polyunsaturated Fat
RDA	Recommended Daily Allowance
RCT	Randomised Controlled Trial
4S	Scandinavian Simvastatin Survival Study
SBP	Systolic Blood Pressure
SFA	Saturated Fat
STARS	St Thomas' Atherosclerosis Regression Study
TG	Triglyceride
tHcy	Total Homocysteine
TOHP	Trials of Hypertension Prevention
TONE	Trial of Nonpharmacologic Interventions in the Elderly
USDA	United States Department of Agriculture
UK	United Kingdom
US	United States of America
VLED	Very Low Energy Diet
VLDL-C	Very Low Density Lipoprotein Cholesterol
WC	Waist Circumference
WHO	World Health Organisation
WHR	Waist to Hip Ratio
WOSCOPS	West of Scotland Coronary Prevention Study

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