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Epidemiology and the role of antioxidants in preventing coronary heart disease: a brief overview

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Coronary heart disease (CHD) is the principal cause of premature death in the Western world. Early epidemiological studies have implicated dietary saturated fat, presumably due to its serum cholesterol-raising effect, as an important risk factor. However, cholesterol and other classical risk factors, such as blood pressure, do not fully explain an individual's risk of CHD (Heller *et al.* 1984).

The theory that the balance between factors that promote free-radical-mediated oxidation and the antioxidant defence mechanisms (i.e. 'oxidative stress') may be a specific risk factor for CHD (Steinberg *et al.* 1989) has gained more acceptance. The aim of the present brief review is to examine critically the recent epidemiological evidence.

ATHEROSCLEROSIS AND LIPID PEROXIDATION *IN VIVO*

It is not the purpose of the present mini-review to examine all direct and indirect evidence in the greatest possible detail. Nevertheless, when considering the possible role of antioxidant vitamins in the prevention of atherosclerosis an appreciation of the fact that not all low-density lipoproteins (LDL) are equally atherogenic is required. LDL particles require some oxidative modification before they become truly atherogenic, vasoconstrictive and enhance platelet aggregation and, thus, thrombosis. Most of the evidence is derived from artificially oxidized lipoprotein particles *in vitro* or from experiments using so-called 'specific' drugs with antioxidant properties.

The difficulty in testing this hypothesis is that highly reactive free radicals have a short half-life and there are several defence mechanisms (superoxide dismutase (*EC* 1.15.1.1), glutathione peroxidase (*EC* 1.11.1.9) and dietary antioxidant vitamins (vitamins C and E or β-carotene) and polyphenols) which can terminate free-radical chain reactions in the water or lipid phase. Methods employing chemical free-radical spin-traps cannot be used in clinical settings, and give little or no information about the nature of the reactive species in animal studies *in vivo*. In addition, the possibility of artefactual formation of lipid peroxides *ex vivo* presents a serious methodological problem.

Yet there is evidence, some of it more convincing than the rest, that lipid peroxides occur in atherosclerotic lesions and perhaps even in plasma. Levels of thiobarbituric acid reactive substances (TBARS), such as malondialdehyde, formed from lipid peroxides are higher in patients with acute myocardial infarction than in healthy controls (Stringer *et al.* 1989). However, the use of TBARS as a measure of *in vivo* lipid peroxidation has fallen into disrepute, due to its lack of specificity and the formation of aldehydic material formed during the test. Coronary atherosclerosis in Swedish patients with a first acute myocardial infarction is more severe in those in whom LDL-lipid peroxidation is readily induced *in vitro* (Regnström *et al.* 1992). Unfortunately, neither the levels of antioxidant vitamins nor those of polyunsaturated fatty acids, the substrate for lipid peroxidation,

Table 1. *Characteristics and strength of epidemiological evidence*

Type of study	Strength
Cross-cultural	+/−
Cross-sectional:	
Healthy subjects	+
Case–control	++
Longitudinal	+++
Intervention	++++

were measured in that study. Avogaro *et al.* (1988) isolated plasma 'oxidized' or 'oxidatively modified' LDL with its characteristically increased negative charge during electrophoresis. In his hands the composition of this fraction differs from LDL oxidized *in vitro*. Re-isolation of native (i.e. not oxidized) LDL does not yield a similar fraction with increased electrophoretic mobility. The presence of 'oxidized' LDL in plasma does not imply the site of formation, a fact recognized by these authors. They suggest that these modified particles may be in process of reverse transport of oxidized lipids to the liver. LDL isolated with gentle procedures from atherosclerotic lesions has many of the characteristics of LDL oxidized *in vitro* (Ylä-Herttuala *et al.* 1989). Antibodies against epitopes of oxidized LDL recognize material in human (and rabbit) atherosclerotic lesions (Palinski *et al.* 1989; Ylä-Herttuala *et al.* 1990), and are localized at sites where macrophage derived foam cells are found. It concerns, however, observations in a relatively small number of patients. More convincing is perhaps the presence of auto-antibodies against oxidized LDL in human serum (Palinski *et al.* 1989; Salonen *et al.* 1992). These titres are higher in patients with carotid atherosclerosis than in controls (Salonen *et al.* 1992). A relationship between auto-antibody titres and smoking was observed. The titres were also related to the progression of the carotid lesions over a 2-year period, suggesting that they reflect 'active' arterial disease.

EPIDEMIOLOGICAL EVIDENCE

The nature of epidemiological studies examining the relationship between dietary and plasma antioxidant vitamins and CHD varies considerably. On the one hand there are large surveys in which information is almost entirely obtained by self-administered questionnaire. Other studies, with actual plasma or tissue antioxidant levels as well as measures of classical risk factors, tend to be relatively small. Epidemiological studies also differ in other aspects (Table 1). Thus, differences in risk factors in population samples of various countries, although related to CHD mortality rates, could be due to many other factors, whether known or not. As in cross-sectional studies of healthy populations, perhaps only 30% may develop CHD and then many years later. Comparisons of risk factors between cases and apparently healthy controls does not suffer from uncertainty in case definition, but subclinical CHD may be prevalent in many controls, thereby reducing the overall contrast between the two groups. Case fatality and disease modification of risk factor levels during the acute phase could seriously affect the results of these studies too. Long-term follow-up of healthy subjects with known risk factor levels is probably the most feasible design provided risk factors are stable. The ultimate

test is whether CHD can be prevented by long-term intervention. Therefore, it should not surprise anyone that the strength of the evidence ranges from the plausible to almost certain proof.

The view that fruit and vegetables protect against coronary or cerebro-vascular disease is not new. Regional standardized mortality ratios of CHD in the UK relate inversely to calculated vitamin C intake, reflecting a long-established regional gradient in lifestyle and social circumstances (Knox *et al.* 1973). In Scotland, where CHD mortality is high, little fruit and green vegetables are eaten (Smith *et al.* 1989).

CROSS-CULTURAL AND CROSS-SECTIONAL STUDIES

Plasma antioxidant vitamins C and E and carotene (predominantly β-carotene) were measured in four randomly-selected populations from North Karelia, South West Finland, Scotland and Italy with differing CHD mortality rates (Riemersma *et al.* 1990). Centralized laboratory analysis was carried out to prevent systematic errors. No consistent relationship with CHD was observed, for instance carotene was low in Scotland in comparison with Italy and Finland. Furthermore, levels did not differ between the high and moderately high CHD areas in Finland. Vitamin E levels differed between the four areas. After adjustment for differing cholesterol levels, low vitamin E was a common feature in Northern Europe but failed to explain the gradients in CHD mortality. This study was expanded to sixteen cohorts (Gey *et al.* 1991). Vitamins C and E (cholesterol-adjusted) were both inversely related to regional CHD mortality rates $r - 0{\cdot}79$ and $r - 0{\cdot}36$. The relationship between CHD and carotene was weaker. After adjustment for differences in classical risk factors such as serum cholesterol and diastolic blood pressure in multivariate analysis only the relationship between low vitamin E levels and CHD mortality remained significant. The relationship with carotene and vitamin C was significant when the results from the Finnish populations were omitted in subsequent analysis of the same data (Gey *et al.* 1993). It was not possible to exclude the confounding influence of dietary linoleic acid, which in itself is inversely related to the risk of CHD (Oliver *et al.* 1990).

Vitamin C levels in leucocytes are low in patients with an acute myocardial infarction (Hulme *et al.* 1972). An acute-phase response is the most likely explanation for this observation as all antioxidant levels are very low during the first days after the heart attack (R. A. Riemersma, unpublished results). Men with significant coronary artery obstructions and regional wall kinetic abnormalities had lower leucocyte vitamin C levels than those with normal arteriograms, irrespective of smoking status (Ramirez & Flowers, 1980). On the other hand neither plasma vitamin C nor cholesterol-adjusted vitamin E differed between those with and without CHD defined on the basis of symptoms, a history of CHD, or objective evidence of ischaemia on a bicycle ergometer exercise test (Salonen *et al.* 1988). Antioxidant vitamins C and E as well as carotene were lower in new cases of angina discovered by screening a large population sample in Edinburgh (Riemersma *et al.* 1991). The odds ratio for angina for men whose levels fell in the lowest quintile relative to the highest quintile of the normal distribution of plasma vitamin E in Edinburgh was 2·51 (confidence intervals (CI) 1·24–5·10). The odds ratio rose to 2·68 (CI 1·07–6.70) after adjustment for total cholesterol and other classical risk factors and the overall trend was significant (P=0·02). The unadjusted odds ratios for angina subjects with lowest concentrations of plasma vitamin C and of carotene were

2·35 (CI 1·16–4·78) and 2·64 (CI 1·32–5·29) respectively. The strength of these inverse relationships was reduced and they were no longer significant after adjustment for smoking and other risk factors. Thus, low plasma levels of vitamins C and E (and diets poor in fresh fruit and green vegetables) are associated with a higher risk of CHD. Such diets are more common in areas of material deprivation.

We have previously reported an inverse relationship between adipose linoleic acid and the risk of angina in this population (Wood *et al.* 1987). Plasma vitamin E is related to adipose linoleate levels (r 0·31, $P<0·001$). The inverse relationship between plasma vitamin E and the likelihood of angina was examined, therefore, in relation to this fatty acid. When it was included in the logistic analysis for vitamin E, adipose linoleate made an independent contribution to the explanation of angina ($P<0·01$), but vitamin E did not ($P<0·09$). The fact that these factors are almost independent of each other is remarkable in view of their common food source. The reasons are not understood but could possibly be due to the fact that antioxidants are more easily destroyed by food processing than polyunsaturated fatty acids (Esterbauer *et al.* 1987). The relationship between vitamin E and carotene and a first acute myocardial infarction has been re-examined in a multicentre case–control study (Kardinaal *et al.* 1993). Adipose tissue levels were used to avoid the acute-phase problem. Carotene but not vitamin E was inversely related to the risk of acute infarction (relative risk 1·89, 95% CI 1·14–3·13, $P<0·05$), independently of history of hypertension, total cholesterol and smoking habit. However, it appears that there are regional differences in Europe since low vitamin E was associated with increased risk of acute myocardial infarction in Scotland (R. A. Riemersma, unpublished results).

LONGITUDINAL STUDIES

Prospective studies of the relationship between vitamin E levels and CHD mortality in individuals have found no significant relationships (Salonen *et al.* 1985; Kok *et al.* 1987). In these studies vitamin E levels were measured in samples stored long term. Levels were very low suggesting that significant losses had occurred, making the results doubtful. The Basle Prospective study of 3000 pharmaceutical employees did not suffer from this problem, as all vitamin measurements (vitamins C and E, carotene) were measured on entry into the study. The relative risk of ischaemic heart disease for men with low plasma carotene levels (<0·23 μM) was 1·53 (CI 1·07–2·20, $P=0·024$; Gey *et al.* 1987). The risk for men with low vitamin C was 25% higher, but this was not significant. Few men had vitamin E levels below the 'optimal' target of 27·5–30 μM and according to the authors this did not lend itself to evaluate whether low vitamin E predisposes to CHD. However, the optimal level is apparently determined from observed relationships with disease or mortality and lacks a firm theoretical basis that justifies a threshold effect. What is important is that all these studies were undertaken at a time when dietary advice to reduce CHD did not consider a role of antioxidants.

Another study used a 24 h dietary recall method to calculate vitamin C intake in Swedish women initially free of CHD. There was no relationship between dietary vitamin C and vascular disease (myocardial infarction, stroke and death; Lapidus *et al.* 1986). Several recent studies all conducted in the USA have re-examined this. Men who regularly take vitamin C supplements had almost 40% reduction in age-standardized mortality from CHD (Enström *et al.* 1992). In this study the relationship to vitamin E

was not considered, but in two subsequent studies from Harvard using food-frequency questionnaires it was considered. Male health professionals and female nurses taking large vitamin E supplements had fewer coronary events (Rimm *et al.* 1993; Stampfer *et al.* 1993) irrespective of other antioxidants (vitamin C and β-carotene). A 29 and 17% reduction in coronary events was observed in doctors with the highest β-carotene and vitamin C intake, although it was only significant in the former. The relationships for vitamin C and β-carotene were not examined separately in the nurses' study. Vitamin E intake without supplements is poorly related to total dietary vitamin E. Men with the highest dietary vitamin E had a reduced relative risk of coronary events but the trend for an association was not significant ($P=0{\cdot}11$). In contrast almost all β-carotene originates from dietary sources in this study and, therefore, the reduced risk provided by β-carotene is of greater significance for public health.

However, there are important limitations to all these studies. American men and women who regularly take large vitamin supplements differ from those who do not take supplements in terms of education, smoking habit, physical activity, use of aspirin. Thus, it appears that vitamin supplements are closely related to lifestyle and it may not be possible to correct for the many confounding factors that this entails. The question remains whether these questionnaires classify total dietary vitamin use of subjects accurately. Alternatively it may not be the dietary intake that matters if other factors significantly modify tissue, plasma or LDL levels (smoking, dietary fat, bioavailability, or perhaps even genetic factors, etc.).

INTERVENTION TRIALS

To date the results of one intervention trial with 25 mg β-carotene have been published in the form of an abstract. In this study β-carotene was associated with a reduced incidence of coronary events but the number of cases was very small (Gaziano *et al.* 1990). The results of one study of the effect of 50 mg vitamin E or 25 mg β-carotene either alone or in combination in smokers should become available soon (ATBC trial). Several other studies are planned and the tendency is to use large supplements that bear no resemblance to dietary intake, a trend no doubt encouraged by the lack of toxicity of vitamins E and C and β-carotene. Of course this may increase the likelihood of a significant result, but the importance of dietary antioxidants in the prevention of CHD will not be answered.

CONCLUSION

Experimental studies show that antioxidants in general can prevent aortic atherosclerotic lesions and argue strongly in favour of a protective role of antioxidant vitamins in the development of atherosclerosis. However, the risk factors for aortic and coronary atherosclerosis are not identical and coronary atherosclerosis is only one aspect of CHD.

This review largely concentrated on the epidemiological evidence. The data suggest an inverse association between CHD mortality and antioxidant vitamins C and E, as well as carotene. However, this relationship is not observed in all countries studied. In Scotland a consistent relationship is observed between low levels of plasma and adipose vitamin E and risk of CHD. Other studies, from the USA, suggest a benefit from high intakes of vitamins C and E in doses much larger than necessary to prevent deficiency diseases. But

these equally point at lifestyle as a recognized factor for the development of CHD. Formal double-blind intervention trials using vitamins C or E have not been conducted and tend to use doses that bear no resemblance to dietary levels of antioxidant vitamins. Firm dietary recommendations to prevent CHD cannot be given on the basis of current evidence.

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