Fruit, Vegetables and Antioxidants: Their Role in the Prevention of Cardiovascular and Other Diseases

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Introduction

Hypercholesterolaemia is universally accepted as a major risk factor for atherosclerosis. However, at any given concentration of blood cholesterol, there is still great variability in the risk of cardiovascular events. One of the major breakthroughs in atherogenesis research has been the realization that oxidative modification of LDL may be a critically important step in the development of the atherosclerotic plaque (Fig. 17.1). The formation of foam cells from monocyte-derived macrophages in early atherosclerotic lesions is not induced by native LDL but only by LDL modifications such as oxidation (Witztum and Steinberg, 1991).

Evidence for LDL oxidation *in vivo* is now overwhelming. In immunocytochemical studies, antibodies against oxidized LDL stain atherosclerotic lesions but not normal arterial tissue (Palinski *et al*., 1989). LDL extracted from animal and human lesions has been shown to be oxidized and is rapidly taken up by macrophage scavenger receptors (Yla-Herttuala *et al*., 1989). In young myocardial infarction (MI) survivors, an association has

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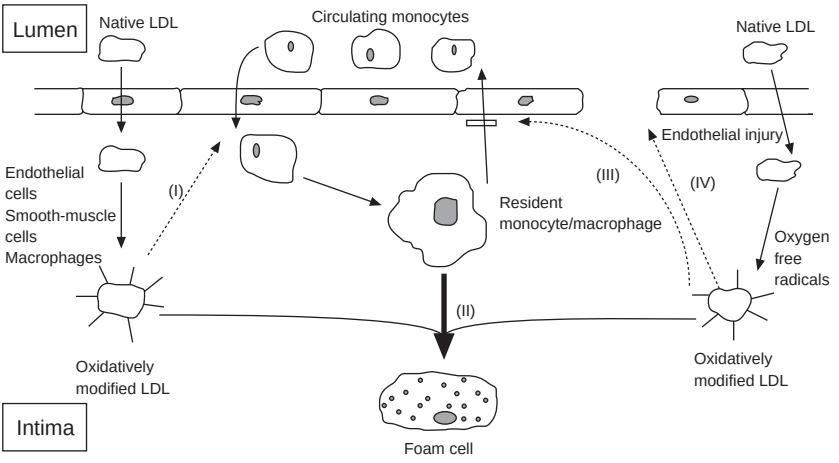

Fig. 17.1. Mechanisms by which oxidation of LDL may contribute to atherogenesis. Oxidized LDL is chemotactic for circulating monocytes (I). These are phenotypically modified and become macrophages. Oxidized LDL is recognized by the scavenger receptor on the macrophage and becomes internalized rapidly. As more lipid is ingested by the macrophage, a foam cell is formed (II). This eventually bursts and a fatty streak, the first phase of an atherosclerotic lesion, results. Oxidized LDL inhibits the motility of resident macrophages and therefore their ability to leave the intima (III). Oxidized LDL is cytotoxic to endothelial cells, leading directly to endothelial cell damage (IV). Oxidation can occur via the effects of reactive oxygen species or due to the oxidation of the cell's own lipids. Adapted from Steinberg *et al.* (1989).

been demonstrated between increased susceptibility of LDL to oxidation and the degree of coronary atherosclerosis (Regnstrom *et al.*, 1992), while the presence of ceroid, a product of lipid peroxidation, has been shown in advanced atherosclerotic plaques (Ball *et al.*, 1987).

Antioxidants and LDL Oxidation

The role of dietary factors in protecting against the change from native to oxidized LDL has received considerable attention. An overview of epidemiological research suggests that individuals with the highest intakes of antioxidant vitamins, whether through diet or supplements, tend to experience 20–40% lower risk of coronary heart disease (CHD) than those with the lowest intake or blood levels (Gaziano, 1994; also see Chapter 13). Vitamin E is the major lipid-soluble antioxidant present in LDL preventing the formation of lipid hydroperoxides from polyunsaturated fatty acids. Vitamin C can scavenge free radicals in the cytoplasm and may also regenerate vitamin E (Leake, 1993). β-Carotene, a vitamin A precursor, does

not have a confirmed antioxidant mechanism (Frei, 1995), although it is contained within LDL (Duthie and Wahle, 1990).

The antioxidant vitamins cannot be synthesized from simple precursors. They are derived mainly from fresh fruit and vegetables. Vitamin E is obtained from vegetable oil and polyunsaturated margarine. In addition, it is sometimes added to margarine as an antioxidant. Thus, concentrations of these vitamins in blood and body tissues are determined by dietary intake, absorption, metabolism and storage.

A number of studies have evaluated the effects of vitamin E on copper-catalysed LDL oxidation in healthy volunteers. Men supplemented with 268, 537 or 805 mg of vitamin E per day for 8 weeks showed a decreased susceptibility of LDL to oxidation. There was no significant effect of daily supplementation with 40 or 134 mg (Jialal and Grundy, 1992). In a study of the effects of low-dose vitamin E supplementation (100 mg day^{-1} for 1 week, then 200 mg day^{-1} for 3 weeks), there was a significant increase in lag time before the onset of LDL oxidation and a significant decrease in the propagation rate (Suzukawa *et al.*, 1995). Princen *et al.* (1995) have evaluated the minimal supplementary dose of vitamin E necessary to protect LDL against oxidation *in vitro* in healthy young adults with a stepwise increase of vitamin E supplements. Resistance of LDL to oxidation increased in a dose-dependent manner, with resistance time differing significantly from baseline even after ingestion of only 17 mg day^{-1} of vitamin E. However, the propagation of lipid peroxidation in LDL was only reduced after intake of 268 and 536 mg day^{-1}.

The effect of high-dose vitamin C supplementation (1000 mg day^{-1}) on LDL oxidation was evaluated in a study of 19 smokers. The vitamin C-supplemented group had a significant reduction in the susceptibility of LDL to oxidation after 4 weeks (Fuller *et al.*, 1996). Jialal *et al.* (1991) found that β-carotene (1–2 μmol l^{-1}) inhibited the oxidative modification of LDL *in vitro* in healthy subjects. However, when the effectiveness of β-carotene, vitamin C and vitamin E supplements was assessed by Reaven *et al.* (1993), susceptibility of LDL to oxidation did not change during β-carotene supplementation (60 mg day^{-1}) but decreased by 30–40% with the addition of vitamin E (1600 mg day^{-1}). Addition of vitamin C (2 g day^{-1}) did not further reduce the susceptibility to oxidation. In a group of smokers and non-smokers given vitamin E supplementation (671 mg day^{-1} for 7 days), resistance of LDL to oxidation increased significantly and the rate of LDL oxidation decreased significantly. There was a small but significant increase in resistance of LDL to oxidation in smokers after supplementation with β-carotene (20 mg day^{-1} for 2 weeks, then 40 mg day^{-1} for 12 weeks), but not when the β-carotene-supplemented group was compared as a whole with the placebo group (Princen *et al.*, 1992). Finally, in a study that evaluated the effects of supplements of β-carotene (30 mg day^{-1}), vitamin C (1 g day^{-1}) and vitamin E (530 mg day^{-1}) for 3 months in men, there was a twofold prolongation of the lag phase of LDL

oxidation and a 40% reduction in the oxidation rate (Jialal and Grundy, 1993). Similarly, we have recently shown that a combination of low-dose antioxidant vitamins (150 mg ascorbic acid, 67 mg α-tocopherol, 9 mg β-carotene daily) over a period of 8 weeks significantly prolonged the lag time to oxidation (Woodside *et al.*, 1997). In the study by Jialal and Grundy (1993), the effects of combined antioxidant supplementation were not significantly different from the effects of vitamin E supplementation alone.

In summary, experimental evidence suggests that antioxidant vitamins can reduce the susceptibility of LDL to oxidation *in vitro*. Vitamin E would appear to be the most effective antioxidant; both β-carotene and vitamin C have produced extensions in lag time to oxidation only in a minority of studies, although it remains possible that they may have a beneficial effect in individuals with poor baseline status.

Antioxidants and CVD: Intervention Studies

Largely negative results have been produced from intervention studies using antioxidant vitamin supplementation with a clinical endpoint such as a cardiovascular event. These have been considered in Chapter 13. A number of reasons have been put forward for these negative findings. One possibility is that the complex mixture of antioxidant micronutrients found in a diet high in fruit and vegetables may be more effective than large doses of one or two antioxidant vitamins.

There are several trials underway at present to assess the effects of antioxidant vitamin supplementation on cardiovascular disease (CVD). In each of these trials, doses of vitamin E greater than 200 mg day^{-1} are being used, and these should be sufficient to increase serum levels at least two- to threefold. The Women's Health Study is a primary prevention trial investigating the effects of vitamin E, β-carotene and aspirin on CVD and cancer in 40,000 women aged 50 years and over (Buring and Hennekens, 1992). In France, the Supplementation Vitamins, Minerals and Antioxidant (SU.VI.MAX) Trial is testing a combination of antioxidant vitamins including vitamin E, vitamin C and β-carotene in 15,000 healthy men and women (Gaziano, 1994). The Heart Protection Study is investigating the effects of vitamins E and C, and β-carotene in 18,000 subjects with above average risk of future MI (Sleight, 1995) and a secondary prevention trial using the same three vitamins in 8000 women has been established in the USA (Women's Antioxidant Cardiovascular Disease Trial, WACDT) (Manson *et al.*, 1995). The Heart Outcomes Protection Study is also assessing vitamin E in 9000 persons with previous MI, stroke or peripheral vascular disease or who have diabetes (HOPE Investigators, 1995). Finally, the GISSI Prevention Trial in Italy is evaluating vitamin E and fish oil supplements among 11,000 patients after a recent MI (Jha *et al.*, 1995). The results of these trials should provide

better evidence about the efficacy and safety of the various antioxidant vitamins.

A trial using supplements of fruit and vegetables would also be useful as the results of the intervention trials so far suggest that any reduction in the risk of disease associated with high antioxidant intake may result from consuming a mix of foods rich in antioxidants rather than consuming antioxidants as single nutrients. A diet rich in fruit and vegetables may represent a more general, favourable promoter of reduced disease risk. Quantification of the relationship between fruit and vegetable intake and cardiovascular risk is important for public health since practical perspectives can be opened for prevention and health education even in the absence of a precise understanding of the underlying mechanism.

Effects of Fruit and Vegetables on Disease

Research examining the effects of a diet rich in fruits and vegetables on disease has been carried out using several types of study.

Observational studies of fruit and vegetable intake

A number of studies of people who eat a diet rich in fruit and vegetables, and therefore rich in antioxidant nutrients, have tried to test the hypothesis that fruit and vegetables lower the risk of CHD (Phillips *et al*., 1980; Chang-Claude *et al*., 1992; Thorogood *et al*., 1994; Key *et al*., 1996). In general, observational studies of vegetarians and those with diets rich in fruits and vegetables support the hypothesis that such diets might lower the risk of CHD. Vegetarians generally have high intakes of cereals, nuts and vegetable oils, carrots and green vegetables as well as fruit. However, vegetarians differ from the rest of the population in a number of important ways: they tend to smoke less, have a lower body mass index and alcohol intake, and come predominantly from higher social classes, all of which are known to confer a health advantage.

Dietary intervention trials

The Woman's Health Initiative (WHI) is currently evaluating the effectiveness of a dietary modification strategy to reduce the incidence of breast and colorectal cancer and CHD (Eaker and Hahn, 1994). The dietary goals of the WHI are multifactorial and include a reduction of total dietary fat intake to 20% of total energy intake, a reduction of saturated fat intake to 7% of total energy intake, and an increase in the intake of fruit and vegetables (five or more daily servings) and grain products. The effects of fat reduction on disease incidence will be hard to differentiate from the effect of an increase in fruit and vegetable consumption.

Dietary intervention trials and secondary prevention

Clinical trials using dietary intervention with clinical endpoints are, up to the present, from secondary prevention. In a study using an α-linolenic acid-rich diet patients were randomly assigned after first MI to the experimental (n = 302) or control group (n = 303) (de Lorgeril *et al*., 1994; Renaud *et al*., 1995). Subjects in the experimental group were advised to eat more bread, more vegetables and legumes, more fish, less meat (beef, lamb and pork), it being replaced by poultry, to have no day without fruit, and to replace butter and cream with a margarine comparable with olive oil. The experimental group consumed considerably less lipids, saturated fat, cholesterol and linoleic acid, but more oleic acid and α-linolenic acid and had increased plasma concentrations of these nutrients and also of vitamins C and E. The diet was also rich in folic acid. After follow-up of 27 months those in the experimental group had a relative risk of 0.27 (95% CI 0.12–0.59, P = 0.001) of MI or cardiac death compared to the control group. These changes were not related to change in cholesterol levels, but to changes in serum fatty acids and an increase in vitamins C and E. A similar study, but in a very different population from India, also produced a fall in mortality after acute MI in patients following a fat-reduced diet rich in soluble dietary fibre and antioxidant vitamins (Singh *et al*., 1992a). Both placebo and intervention groups were advised to follow a fat-reduced diet but the intervention group were also advised to eat more fruit, vegetables, nuts and grain products.

The feasibility of a long-term randomized study of a diet rich in vegetables to prevent recurrence of breast cancer has been examined (Pierce *et al*., 1997). The daily diet consisted of five vegetable servings, 16 ounces of fresh vegetable juice, three fruit servings, 15% of energy from fat and 30 g fibre. The juice was added in order to increase micronutrient intake without adding bulk and potential discomfort associated with high fibre intakes.

Therefore, although intervention trials using antioxidant supplements are important in evaluating possible beneficial effects of antioxidants against development or progression of CVD, they have limitations and should be considered as only one component in the totality of available research evidence. It may be that a lifetime of intake is required to show a protective effect, or that a mixture of natural antioxidants, including other bioactive compounds in addition to vitamins C, E and β-carotene, found in fruits and vegetables provide the necessary protective mixture. Further study using diets rich in fruit and vegetables should provide evidence for their beneficial effect on disease.

Effects of Fruit and Vegetables: Biochemical Observations

A recent study asked subjects with normal lipid concentrations who ate three or fewer servings of fruit and vegetables daily to consume eight

servings per day (Zino *et al*., 1997). Plasma concentrations of vitamin C, retinol, α-tocopherol, α- and β-carotene, lipids and lipoproteins were assessed before and after an 8-week intervention period. The plasma vitamin C, α-carotene, and β-carotene concentrations increased, while concentrations of retinol, α-tocopherol, lipids and lipoproteins remained unchanged despite some increase in dietary vitamin E and a small reduction in saturated fat intake. An interesting addition to the results would have been the inclusion of data on the susceptibility of LDL to oxidation. The authors concluded that more specific dietary advice to modify fat intake may be necessary to reduce the risk of CVD.

By contrast, Singh *et al*. (1992b) found over a 12-week period that fruit and vegetable administration in subjects at high risk of CHD lowered total- and LDL-cholesterol and triglyceride levels, and increased HDL-cholesterol (see Chapter 16). Another study by Wise *et al*. (1996) using dehydrated fruit and vegetable extracts over a period of 28 days in 15 healthy adults aged 18–53 years produced increases of the order of 50- to 2000-fold in carotenoid and tocopherol levels, while serum lipid peroxides decreased fourfold during the intervention period, with much of this lowering taking place during the first week.

Dietary Supply of Antioxidants in the Developed World

Vitamin C and β-carotene are available from fruits and/or vegetables, and vitamin E from vegetable oils. Southern European countries consuming the classical Mediterranean diet show that optimal plasma levels of these antioxidants can easily be achieved. This diet is characterized by a preference for fresh products and frequent consumption of fruit/vegetables/legumes and oils with high vitamin E content. In contrast, major parts of populations in the USA or in northern parts of Europe do not consume optimal amounts of antioxidant nutrients. The availability of lower-priced convenience foods in the USA acts against the consumption of freshly prepared foods. Thus, only 10% of Americans achieved five servings of fruit and vegetables daily (Patterson *et al*., 1990) as recommended by the United States' national food guide, the Food Guide Pyramid (Achterberg *et al*., 1994). Only a quarter consumed fruits or vegetables rich in vitamin C or the carotenoids, and 41% had no fruits or vegetables on the day the survey was carried out (Block, 1991). A recent re-analysis of the NHANES II data showed that vitamin supplements are the major contributors of the principal antioxidant micronutrients in the US diet (28% of vitamin C and 46% of vitamin E) (Block *et al*., 1994).

The requirement for a micronutrient is the minimum quantity necessary to prevent a deficiency. The translation of minimum requirement to a dietary recommendation for population groups necessitates that allowance be made for a number of variables, including: (i) periods of low intake, (ii)

increased utilization, (iii) individual variability, and (iv) bioavailability. In the United States, the recommended daily allowances (RDAs) incorporate 'margins of safety' intended to be sufficiently generous to encompass the variability in the minimum requirement among people, and bioavailability from different food sources.

To date, recommendations on micronutrient intake have therefore primarily been intended to prevent clinically overt deficiencies, e.g. scurvy or neural tube defects. If, however, observational and experimental evidence continues to show that the prevention of slow multistage processes such as CVD and cancer might require a higher intake of some essential antioxidants, then a recommended intake should be devised referring to amounts considered sufficient for the avoidance of these disease states. Gey (1995) suggests that the present recommendations will require either an upgrading or an additional term, e.g. a recommended optimum intake (ROI) which will vary with gender, age, with special requirements for smokers, pregnancy and the elderly. The ROI could be defined as sufficient (culture- and/or region-specific) intake to achieve blood levels associated with the observed minimum relative risk of disease (Gey, 1995). A ROI would simply quantify specific dietary constituents of conceivably crucial importance within the still desirable 'five servings of fruit and vegetables daily.'

Conclusion

There is strong scientific evidence to support an increase in intakes of vegetables and fruit in the prevention of disease. Further research is required to clarify which particular components of fruit and vegetables are responsible for their protective effects. Antioxidants have a definite protective effect on the susceptibility of LDL to oxidation, but the relevance of this to CHD incidence, and the possibility of other important bioactive micronutrients in vegetables and fruit requires further research. Only then can dietary recommendations, referring to intakes of these specific micronutrients required to avoid disease in addition to the general 'five servings a day', be made possible.

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