Antioxidants and Their Role in Coronary Heart Disease Prevention

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Introduction

One hundred and fifty years have elapsed since Vogel identified cholesterol as a component of the atherosclerotic plaque (Vogel, 1847). Over 80 years have passed since Anitschkow (1913) demonstrated the atherogenic potential of dietary cholesterol. The presence of cholesterol and its oxidized derivatives in arterial deposits was demonstrated early this century. The nature of the initiating event(s) is part of the more recent history of this disease.

The work pioneered by Gofman and his colleagues (1950) demonstrated that cholesterol and other lipids were transported in the blood as a lipid–protein continuum which could be separated into classes of lipoproteins based on their hydrated densities. The class of lipoproteins identified as LDL (low-density lipoproteins) was shown to be the major transporter of cholesterol and was also shown to be most closely correlated with risk of cardiovascular disease. The lipoprotein story is continuing as can be seen from the discoveries of the extra-atherogenic nature of lipoprotein(a) (Lp(a)) (Berg, 1963) and the finding of a spectrum of LDL aggregates of different sizes and atherogenic potential (Krauss, 1991).

Twenty years ago Davignon (1978) presented a unified picture of the process of atherosclerosis. He showed the participation of environmental influences (diet and smoking, among others), humoral influences (circulating lipoproteins, hormones, etc.) and arterial metabolic effects, all of which combined to form the atherosclerotic plaque. The environmental and

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humoral areas have been worked over pretty thoroughly, although new observations continue to amend the picture. The mode of cholesterol accumulation in the arterial wall is complex and is only now beginning to be understood.

Currently it is believed that blood monocytes adhere to the endothelial lining, migrate through endothelial junctions and differentiate to become macrophages (Ross, 1986). The macrophages take up lipoproteins and become cholesteryl ester-rich foam cells which make up the fatty streak found beneath an unbroken layer of endothelial cells. Foam cells may also arise from smooth muscle cells that migrate into the subendothelial space. The fatty streak proliferates to form complex lesions and fibrous plaques by mechanisms, as yet undelineated, which involve an array of growth factors (Pomerantz *et al*., 1995).

Oxidized Lipid and Atherosclerosis

The macrophages and foam cells can continue to take up LDL. One might expect that incubation of these moieties (monocytes, macrophages, smooth muscle cells) with concentrated sources of cholesterol (LDL) would convert them to foam cells but this is not the case. However, another form of LDL, oxidized LDL, has been identified *in vivo* and has been shown to be taken up when incubated with the various cell types found in atherosclerotic lesions (Henriksen *et al*., 1981). Oxidatively modified LDL is also taken up by the scavenger receptor. This observation is the basis of the oxidized-LDL hypothesis used to explain the early steps in the atherosclerotic process.

The possible involvement of oxidized lipid in the atherosclerotic process was considered long before the experiments cited above. About 50 years ago Dam and his colleagues (Jessen *et al*., 1951; Glavind *et al*., 1952) reported on the isolation of peroxidized lipid from atherosclerotic arteries. Altschul (1946) suggested that heated (i.e. oxidized) cholesterol might be more atherogenic for rabbits than pure cholesterol. Analysis of atherosclerotic arteries carried out in 1943 revealed the presence of cholesta-3,5-diene-7-one, cholesta-4,6-diene-3-one, and 3β, cholestane 3β, 5α, 6β-triol (Hardegger *et al*., 1943).

Imai *et al*. (1976) fed rabbits recrystallized cholesterol or impure cholesterol (USP-cholesterol that had been in their laboratory for some years and had become yellowed and rancid). The aortas were examined by electron microscopy 24 h later. The old cholesterol led to 73% more degenerated cells than the recrystallized material. The major autooxidation products were cholesta-3,5-diene-7-one, 25-hydroxycholesterol, 7-keto-cholesterol, 7α and 7β-hydroxycholesterol and cholestane-3β, 5α, 6β-triol (Peng *et al*., 1978). The oxidation products, especially 25-hydroxycholesterol and the triol, are toxic to cultured rabbit arterial smooth muscle cells (Peng

et al., 1978, 1979) and cause arterial injury to rabbits (Cook and MacDougal, 1968). Cholesterol oxidation products are transported in rabbit lipoproteins (Peng *et al*., 1987).

Comparison of atherogenicity of crystalline and amorpous cholesterol showed the latter to be less cholesterolaemic but more atherogenic. The broad melting point of amorphous cholesterol suggests the presence of impurities (Kritchevsky *et al*., 1969). The angiotoxicity and atherogenicity of cholesterol oxides has been reviewed by Peng *et al*. (1991). Hodis *et al*. (1991) reported that normal rabbit plasma contains 81 μmol l^{-1} of cholesterol oxides. After feeding a high cholesterol diet to rabbits for 6 weeks the level of plasma cholesterol oxides rose by a factor of 5, while total plasma cholesterol rose by a factor of 64. Aortic levels of total cholesterol oxides rose 150%. Several compounds that had not been present in arteries of the control group were isolated from arteries of cholesterol-fed rabbits, namely, cholesterol β-oxide, 7-ketocholesterol and 25-hydroxy-cholesterol. There have been earlier reports of isolation of 26-hydroxy-cholesterol (Smith and vanLier, 1970) and polar sterol esters have been shown to be present in human atherosclerotic plaques (Brooks *et al*., 1971). The data suggest that cholesterol oxidation occurs *in vivo*.

Probucol [4,4′-(isopropylidenedithio)bis(2,6-di-t-butylphenol)] is one of a series of potential antioxidants initially synthesized by chemists at the Ethyl Corporation. It was developed by the Dow Chemical Co. and, under the code number of DH-581, was found to reduce significantly the severity of cholesterol-induced atherosclerosis in rabbits (Kritchevsky *et al*., 1971) and to lower cholesterol levels in humans (Tedeschi *et al*., 1980). Its precise mode of action was unclear (Kritchevsky, 1980) but a role as an antioxidant was not considered. Since it is transported in the lipoproteins of the serum it is in the right place to exert its antioxidant properties.

Steinberg and his colleagues (1989) have conducted a series of elegant studies to demonstrate that LDL oxidation may indeed play a crucial role in atherogenesis. They showed that probucol, a hypocholesterolaemic agent, could prevent oxidation of LDL *in vitro* and *in vivo* and could prevent progression of atherosclerosis in the Watanabe heritable hyperlipidaemic (WHHL) rabbit and that this action was due to its antioxidant properties and independent of its hypolipidaemic properties (Parthasarathy *et al*., 1986; Carew *et al*., 1987). They have also shown that macrophage foam cells isolated from atherosclerotic lesions of rabbits degrade modified lipoproteins and promote oxidation of LDL *in vitro* (Rosenfeld *et al*., 1991). The oxidation hypothesis has been discussed elsewhere in detail (Steinberg *et al*., 1989; Esterbauer *et al*., 1997).

Oxidation of lipoproteins plays a significant role in atherogenesis. That knowledge offers an opportunity to test effects of antioxidants as inhibitors of atherogenesis, which can be done in experimental animals. They can also be studied as inhibitors of lipoprotein oxidation or reducers of risk of cardiovascular disease.

Antioxidants and Arterial Disease

Vitamin E effects on experimental atherosclerosis have been investigated since the 1940s. In early studies conducted using the cholesterol-fed rabbit model, Dam (1994) found that dietary α-tocopherol did not affect atherosclerosis, and Bruger (1945) and Moses *et al*. (1952) reported the intramuscular administration of vitamin E did not inhibit experimentally induced atherosclerosis. More recently, several authors have found that vitamin E may inhibit atherosclerosis in rabbits (Brattsand, 1975; Wilson *et al*., 1978) and chickens (Smith and Kummerow, 1989). Bocan *et al*. (1992), working with rabbits, found antioxidant therapy (vitamins C and E) to alter progression of diet-induced fatty streaks but to have no effect on progression as regression of more complicated iliac femoral lesions. Singh *et al*. (1995) fed rabbits a high-fat diet for 12 weeks then randomized them to treatment with fruits, vegetables and mustard oil, vitamins C, E, and β-carotene, a high-fat diet or a low-fat diet. After 12 weeks, rabbits in the first two groups exhibited significantly lower plaque size in the aorta and coronary arteries than did those fed the unsupplemented diet. Aortic plaque size was fourfold higher in the fat-fed groups and coronary artery plaque size was about double that of the fruit- or vitamin-fed groups. Verlangieri and Bush (1992) studied atherosclerosis progression and regression in the carotid arteries of monkeys using ultrasound. Addition of α-tocopherol to the diet inhibited atherogenesis and enhanced regression. Godfried *et al*. (1989) reported that very high dietary levels of vitamin E potentiated atherosclerotic lesions in rabbits.

In assessing the efficacy of antioxidants *vis-à-vis* their effects on actual coronary events, we can examine epidemiological studies (observational or case–control) or intervention studies. As we will see, the findings are surprisingly different. Gey *et al*. (1991) has attempted to correlate mortality from ischaemic heart disease (IHD) in 16 European populations being examined in the WHO/MONICA Core Study. In the 12 populations with similar blood pressure and plasma cholesterol levels (5.69–6.21 mmol l^{-1}) he found no significant correlation between those parameters and IHD mortality but levels of vitamin E exhibited a strong inverse correlation (P=0.002). When all 16 populations were evaluated, correlation of IHD mortality with serum cholesterol levels was associated moderately, but the correlation with vitamin E levels was much stronger (Gey *et al*., 1991). The protective effects of dietary antioxidants were evaluated in a later study with more subjects and the rating for the individual factors was: vitamin E > carotene = vitamin C > vitamin A (Gey *et al*., 1993a). Gey (1995) has recently reviewed this field in depth.

The effects of vitamin C have also been considered (Frei *et al*., 1989). Enstrom *et al*. (1992) found a strong inverse relationship between vitamin C intake and cardiovascular mortality. They assessed data from over 11,000 people in the NHANES I and the 10-year follow-up. The NHANES was a

national study of diet and health conducted in various populations in the USA. Low plasma levels of vitamin C may increase risk for stroke (Gey *et al*., 1993b). Vitamin C also protects human LDL against atherogenic modification (Retsky *et al*., 1993). A diet high in these substances, especially vitamin C and carotenes, is really a diet high in fruits and vegetables.

There have been a number of observational studies examining dietary antioxidant intakes and risk of coronary disease. In an examination of participants in the LRC-CPPT Study (Lipid Research Clinics Coronary Primary Prevention Trial) a decreased risk of coronary heart disease was seen in participants with higher serum carotenoid levels (Morris *et al*., 1994). Stampfer *et al*. (1993), in analysing dietary and health data from 87,245 female nurses, observed a highly significant trend for coronary risk reduction with increasing vitamin E intake (diet plus supplements). The association was not significant if the contribution of the supplements was not included. Rimm *et al*. (1993) found a similar pattern in 39,910 male health professionals. Namely, there was a highly significant association between trend towards reduction of risk and intake of vitamin E from food plus supplements. When the contributions of either food or supplements alone were analysed neither provided a significant trend. Knekt *et al*. (1994) analysed the correlations between reported antioxidant intake and cardiovascular mortality in 5133 Finnish subjects (2748 men and 2385 women). A significant trend towards protection by vitamin E was only observed in the female cohort. A study of dietary antioxidant intake and carotid artery wall thickness in 11,307 subjects suggested a protective effect for dietary α-tocopherol and vitamin C (Kritchevsky *et al*., 1995). A subsequent examination of this population in which carotid artery plaque was quantitated showed a protective effect of carotenoids (Kritchevsky *et al*., 1995).

In a comparison of 50-year-old men in Sweden and Lithuania, Kristenson *et al*. (1997) found the Swedish cohort, who exhibit one-fourth the heart disease mortality, to be significantly taller, to have lower systolic blood pressure and to be significantly more active physically. Their plasma total and LDL-cholesterol levels were significantly higher. Assay of the plasma concentration of lipid-soluble antioxidants showed the Swedish subjects to have significantly more β-carotene (by 35%), lycopene (by 88%) and α-tocopherol (by 84%). Cleary *et al*. (1997) also found levels of lipophilic antioxidants not to be depleted in men with severe atherosclerosis.

Kushi *et al*. (1996) followed 34,486 post-menopausal women free of coronary disease at the initial screen for 7 years. In that period, 242 of the women (0.70%) died of coronary disease. Intake of vitamin E was associated inversely with risk of death but intake of vitamins A and C was not. Riemersma *et al*. (1991) found plasma concentrations of vitamins C, E and carotene to be associated inversely with risk of angina. After adjustment for smoking only the inverse relationship with vitamin E remained. Adipose tissue levels of β-carotene have been found to be associated with reduced risk of myocardial infarction (MI) but there was no association with α-tocopherol

levels (Kardinaal *et al.*, 1993). In a population-based, nested case–control study Street *et al.* (1994) found that serum levels of a variety of carotenoids were inversely associated with risk of MI, especially among smokers.

One of the early clinical trials involving use of vitamin E was carried out by Gillilan *et al.* (1977) who found it not to be effective in the treatment of angina. Two well-publicized trials of the effect of vitamins E or A and β-carotene on incidence of lung cancer in smokers have also obtained data relating to cardiovascular disease. Omenn *et al.* (1996) found slightly higher cardiovascular mortality in the group given vitamin A and β-carotene supplements. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) Group (1994) found 5% fewer coronary deaths in men who had taken α-tocopherol and 11% more deaths in those who received β-carotene supplements. Subjects who received α-tocopherol exhibited a very slight decrease in incidence of angina pectoris whereas those receiving β-carotene showed a slight increase. Major coronary events were studied during the follow-up period (median 5.3 years). The totals of non-fatal MI and fatal coronary events in the four groups were: α-tocopherol, 94; β-carotene, 113; α-tocopherol and β-carotene, 123; and placebo, 94. The ratio of fatal events to total events was: α-tocopherol, 57.4%; β-carotene, 65.5%; α-tocopherol plus β-carotene, 54.5%; and placebo, 41.5% (Rapola *et al.*, 1996, 1997). Hennekens *et al.* (1996) carried out a randomized, double-blind, placebo-controlled trial of β-carotene in 22,071 male physicians aged 40–84 years. After 12 years they found no difference in the rate of cardiovascular events.

Hodis *et al.* (1995) have demonstrated a reduction in angiographically measured coronary artery disease progression in men given 100 IU or more of vitamin E daily. No benefit was found for supplementary vitamin C. Singh *et al.* (1996) found that a combination of vitamins A, C and E, and β-carotene administered within a few hours after acute MI and continued for 28 days led to significantly fewer cardiac events. Incidence of angina pectoris was significantly lower in the group receiving antioxidants. Cardiac deaths plus non-fatal infarction numbered 13 (20% of subjects) in the patients given antioxidants and 19 (30.6% of subjects) in the control group ($P < 0.05$).

The Cambridge Heart Antioxidant Study (CHAOS) carried out a double-blind, placebo-controlled study in 2002 patients with proven coronary atherosclerosis (Stephens *et al.*, 1996). The test group of 1035 subjects was given α-tocopherol (400 or 800 IU per day) and 967 controls were given a placebo. After 510 days, non-fatal MI numbered 14 in the α-tocopherol group and 41 in the placebo group. MI fatalities were higher in the test group (18 vs. 13) as were total cardiovascular deaths (27 vs. 23). Deaths from all causes were 38% higher in the α-tocopherol group (36 vs. 26). The difference in fatal MI was due, in part, to an excess of early deaths and the authors suggest that α-tocopherol had not yet had time to exert its effects. The treatment had no effect on serum cholesterol.

Greenberg *et al*. (1996) studied mortality associated with low plasma levels of β-carotene and the effect of oral supplementation (50 mg day^{-1}). There were a total of 1720 subjects (1188 men and 532 women). The treatment was carried out for a median period of 4.3 years and the median follow-up was 8.2 years. Subjects whose plasma levels of β-carotene were in the highest quartile at the beginning of the study had the lowest risk of death from all causes compared with those in the lowest quartile. Ingestion of the β-carotene supplement did not reduce all-cause or cardiovascular mortality. Total deaths were 5% higher in the supplemented group (146 vs. 139); cardiovascular deaths were 15% higher in this group (68 vs. 59) and cancer deaths were 14% lower (38 vs. 44).

The effects of another group of dietary antioxidants, flavonoids, on coronary mortality or stroke have also been evaluated. In the Netherlands a group of 552 men was followed between 1970 and 1985. The authors concluded that habitual flavonoid intake might be protective against stroke (Keli *et al*., 1996). In a Finnish study, the total and coronary mortality in men were both higher in the subjects with lowest flavonoid intake than in those in the highest intake (Knekt *et al*., 1996). Relative risk for all-cause mortality was reduced significantly in both men and women. Relative risk for coronary mortality was reduced significantly in women and was reduced from 14.4 to 8.3% in men, but the difference did not reach statistical significance. In the Male Health Professionals Study, on the other hand, the investigators found no strong inverse relationship between total flavonoid intake and coronary disease incidence (Rimm *et al*., 1996).

Dilemma about Antioxidants

We are left with this dilemma – consumption of a diet containing appreciable amounts of antioxidants and high plasma levels of antioxidants appeared to be protective in relation to CHD and yet trials in which selected single antioxidants are added to the diet provide scant and inconsistent evidence of a protective effect. Several possible explanations come to mind. First, consumption of a diet high in vitamins, carotenes, and flavonoids reflects intake of a wide variety of these substances, some of which may still be unidentified. It could be that several of these compounds work in concert whereas single components fed individually are without effect. It is also possible that other dietary components (trace minerals, for instance) may be effectors of carotenoid action. Virtually all nutritional studies are based on replacing one component of the diet with another without considering how these specific substances may interact with the rest of the diet. As an example from fibre research, in an atherogenic rabbit diet in which the fibre is cellulose, casein is more cholesterolaemic and atherogenic than soy protein; when cellulose is replaced by lucerne the two proteins become equivalent (Kritchevsky *et al*., 1977).

Could it be that the intervention trials have not been carried out for sufficiently long periods? Habitual intake of a wide variety of antioxidants provides a greater source of vitamins or carotenoids over a longer time than administration of one or two compounds for a relatively short time to subjects who have already compromised their level of risk by inappropriate behaviour, e.g. smoking.

Lifestyle may be one of the determinants of the picture presented to the investigators. Heart disease and cancer are generally described as lifestyle disease but the research approach focuses on only one aspect of lifestyle. Slattery *et al.* (1995) examined dietary antioxidants and plasma lipids in participants in the Coronary Artery Risk Development in Young Adults (CARDIA) study and found that higher intake of dietary antioxidants is associated with other lifestyle factors such as physical activity and non-smoking. In Britain, plasma concentrations of antioxidants are related to social class, being higher in the better educated and more affluent (Gregory *et al.*, 1990). If protective levels of antioxidants such as carotenoids, tocopherols, and others are part of a life-long behavioural pattern, we cannot expect too much if we influence one aspect of risk by dietary supplements without addressing other behaviours.

Most studies have been carried out in men. Knekt *et al.* (1996) found flavonoid intake to be significantly associated with reduced coronary risk in women. The risk was lowered non-significantly in men. It is well established that pre-menopausal women are relatively protected against coronary disease and that the protection may be due to circulating oestrogens which affect positively the LDL/HDL (high-density lipoprotein) cholesterol ratio. The protection is lost in post-menopausal women. Could antioxidant protection by oestrogen be another factor in lowering risk in young women? Shwaery *et al.* (1997) have reported that 17β-oestradiol protects LDL against copper-mediated oxidation *in vitro*. In another context, we showed many years ago that oestradiol inhibited ascorbic acid-catalysed oxidation of methyl linoleate (Kritchevsky and Tepper, 1964). White *et al.* (1997) have summarized results from other studies in which oestrogens influenced rates of LDL oxidation.

Accumulating data lend validity to the hypothesis involving oxidized LDL as a major factor in atherogenesis and protection being offered by antioxidant vitamins. Questions relating to *in vivo* initiation of LDL oxidation remain to be answered (Halliwell, 1995) and randomized trials with antioxidant vitamins are needed (Hennekens *et al.*, 1995).

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References

The Alpha-Tocopherol Beta-Carotene Cancer Prevention Study Group (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *New England Journal of Medicine* 330, 1029–1035.

Altschul, R. (1946) Experimental cholesterol arteriosclerosis. Changes produced in skeletal muscle. *Archives of Pathology* 42, 277–284.

Anitschkow, N. (1913) Über die veranderung der kaninchenaorta by experimenteller cholesterinsteatose. *Beitrage zur Patholischen Anatomie und zur Allgemeinen Pathologie* 56, 379–404.

Berg, K. (1963) A new serum type system in man: the Lp(a) system. *Acta Pathologica et Microbiologica Scandinavica* 59, 369–382.

Bocan, T.M.A., Mueller, S.B., Brown, E.Q., Uhlendorf, P.D., Mazur, M.J. and Newton, R.S. (1992) Antiatherosclerotic effects of antioxidants are lesion-specific when evaluated in hypercholesterolemic New Zealand White rabbits. *Experimental and Molecular Pathology* 57, 70–83.

Brattsand, R. (1975) Actions of vitamins A and E and some nicotinic acid derivatives on plasma lipids on lipid infiltrations of aorta in cholesterol-fed rabbits. *Atherosclerosis* 22, 47–61.

Brooks, C.J.W., Steel, G., Gilbert, J.D. and Harland, W.A. (1971) Characterization of a new group of polar sterol esters from human atherosclerotic plaques. *Atherosclerosis* 13, 223–237.

Bruger, M. (1945) Experimental atherosclerosis. VII. Effect of vitamin E. *Proceedings of the Society for Experimental Biology and Medicine* 59, 56–57.

Carew, T.E., Schwenke, D.C. and Steinberg, D. (1987) Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants *in vivo* can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Proceedings of the National Academy of Sciences, USA* 84, 7725–7729.

Cleary, J., Mohr, D., Adams, M.R., Celermayer, D.S. and Stocker, R. (1997) Plasma and LDL levels of major lipophilic antioxidants are similar in patients with advanced atherosclerosis and age-matched controls. *Free Radical Research* 26, 175–182.

Cook, R.P. and MacDougal, J.D.B. (1968) Experimental atherosclerosis in rabbits after feeding cholestanetriol. *British Journal of Experimental Pathology* 49, 265–271.

Dam, H. (1944) Ineffectiveness of vitamin E in preventing cholesterol deposition in the aorta. *Journal of Nutrition* 28, 289–295.

Davignon, J. (1978) The lipid hypothesis: pathophysiological basis. *Archives of Surgery* 113, 28–34.

Enstrom, J.E., Kanim, L.E. and Klein, M.A. (1992) Vitamin C intake and mortality among a sample of the United States population. *Epidemiology* 3, 194–202.

Esterbauer, H., Schmidt, R. and Hayn, M. (1997) Relationships among oxidation of low density lipoprotein, antioxidant protection and atherosclerosis. *Advances in Pharmacology* 38, 425–455.

Frei, B., England, L. and Ames, B.N. (1989) Ascorbate is an outstanding antioxidant in human blood plasma. *Proceedings of the National Academy of Sciences, USA* 86, 6377–6381.

Gey, K.F. (1995) Ten-year retrospective on the antioxidant hypothesis of arteriosclerosis. Threshold plasma levels of antioxidant micronutrients related to minimum cardiovascular risk. *Journal of Nutritional Biochemistry* 6, 206–236.

Gey, K.F., Puska, P., Jordan, P. and Moser, U.K. (1991) Inverse correlation between plasma vitamin E and mortality from ischemic heart diease in cross cultural epidemiology. *American Journal of Clinical Nutrition* 53, 326S–334S.

Gey, K.F., Moser, U.K., Jordan, P., Stähelin, H.B., Eichholzer, M. and Lüdin, E. (1993a) Increased risk of cardiovascular disease at suboptimal plasma concentrations of essential antioxidants: an epidemiological update with special attention to carotene and vitamin C. *American Journal of Clinical Nutrition* 57, 787S–797S.

Gey, K.F., Stähelin, H.B. and Eichholzer, M. (1993b) Poor plasma status of carotene and vitamin C is associated with higher mortality from ischemic heart disease and stroke: Basel Prospective Study. *Clinical Investigation* 71, 3–6.

Gillilan, R.E., Mondell, B. and Warbasse, J.R. (1977) Quantitative evaluation of vitamin E in the treatment of angina pectoris. *American Heart Journal* 93, 444–449.

Glavind, J., Hartmann, S., Clemmesen, J., Jessen, K.E. and Dam, H. (1952) Studies on the role of lipoperoxides in human pathology. II. The presence of peroxidized lipids in the atherosclerotic aorta. *Acta Pathologica et Microbiologica Scandinavica* 30, 1–6.

Godfried, S.L., Combs, G.F. Jr, Saroka, J.M. and Dillingham, L.A. (1989) Potentiation of atherosclerotic lesions in rabbits by a high dietary level of vitamin E. *British Journal of Nutrition* 61, 607–617.

Gofman, J.W., Lindgren, F., Elliott, H., Mantz, W., Hewitt, J., Strisower, B., Herring, V. and Lyon, T.P. (1950) The role of lipids and lipoproteins in atherosclerosis. *Science* 111, 166–171.

Greenberg, E.R., Baron, J.A., Karagas, M.R., Stukel, T.A., Merenberg, D.W., Stevens, M.M., Mandel, J.S. and Haile, R.W. (1996) Mortality associated with low plasma concentration of beta carotene and the effect of oral supplementation. *Journal of the American Medical Association* 275, 699–703.

Gregory, J., Foster, K., Tyler, H. and Wiseman, M. (1990) *The Dietary and Nutritional Survey of British Adults*. HMSO, London.

Halliwell, B. (1995) Oxidation of low-density lipoproteins: questions of initiation, propagation and the effect of antioxidants. *American Journal of Clinical Nutrition* 61, 670S–677S.

Hardegger, E., Ruzicka, L. and Tagmann, E. (1943) Untersuchungen über organextrakte. Zur kenntnis der unverseifbaren lipoide aus arteriosklerotischen aorten. *Helvetica Chimrurgica Acta* 30, 2205–2221.

Hennekens, C.H., Gaziano, J.M., Manson, J.E. and Buring, J.E. (1995) Antioxidant vitamin-cardiovascular disease hypothesis is still promising, but still unproven: the need for randomized trials. *American Journal of Clinical Nutrition* 62, 1377S–1380S.

Hennekens, C.H., Buring, J.E., Manson, J.E., Stampfer, M., Rosner, B., Cook, N.R., Belanger, C., La Motte, F., Gaziano, J.M., Ridker, P.M., Willett, W. and Peto, R. (1996) Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *New England Journal of Medicine* 334, 1145–1149.

Henriksen, T., Mahoney, E.M. and Steinberg, D. (1981) Enhanced macrophage degradation by low density lipoprotein previously incubated with cultured endothelial cells: recognition by receptors for acetylated low-density lipoprotein. *Proceedings of the National Academy of Sciences, USA* 78, 6499–6503.

Hodis, H.N., Crawford, D.W. and Sevanian, A. (1991) Cholesterol feeding increases plasma and aortic tissue cholesterol oxide levels in parallel: further evidence for the role of cholesterol oxidation in atherosclerosis. *Atherosclerosis* 89, 117–126.

Hodis, H.N., Mack, W.J., LaBrec, L., Cashin-Hemphill, L., Sevanian, A., Johnson, R. and Azen, S.P. (1995) Serial coronary angiographic evidence that antioxidant vitamin intake reduces progression of coronary artery atherosclerosis. *Journal of the American Medical Association* 273, 1849–1854.

Imai, H., Werthessen, N.T., Taylor, C.B. and Lee, K.T. (1976) Angiotoxicity and atherosclerosis due to contaminants of U.S.P. grade cholesterol. *Archives of Pathology and Laboratory Medicine* 100, 565–572.

Jessen, K.E., Glavind, J., Hartmann, S. and Dam, H. (1951) Peroxidation of human adipose in peripheral venous disease. *Acta Pathologica et Microbiologica Scandinavica* 29, 73–76.

Kardinaal, A.F.M., Kok, F.J., Ringstad, J., Gomez-Aracena, J., Mazaev, V.P., Kohlmeier, L., Martin, B.C., Aro, A., Karle, J.D., Delgado-Rodriquez, M., Riemersma, R.A., van't Veer, P., Huttunen, J.K. and Martin-Moreno, J.M. (1993) Antioxidants in adipose tissue and risk of myocardial infarction: the EURAMIC Study. *Lancet* 342, 1379–1384.

Keli, S.O., Hertog, M.G.L., Feskens, E.J.M. and Kromhout, D. (1996) Dietary flavonoids, antioxidant vitamins and incidence of stroke. *Archives of Internal Medicine* 154, 637–642.

Knekt, P., Reunanen, A., Jarvinen, R., Seppanen, R., Helivaara, M. and Aromaa, A. (1994) Antioxidant vitamin intake and coronary mortality in a longitudinal population study. *American Journal of Epidemiology* 139, 1180–1189.

Knekt, P., Jarvinen, R., Reunanen, A. and Maatela, J. (1996) Flavonoid intake and coronary mortality in Finland: a cohort study. *British Medical Journal* 312, 478–481.

Krauss, R.M. (1991) Low density lipoprotein subclasses and risk of coronary artery disease. *Current Opinions in Lipidology* 2, 248–252.

Kristenson, M., Zieden, B., Kucinskienë, Z., Schäfer-Elender, L., Bergdahl, B., Elwing, B., Abaravicus, A., Razinkovenë, L., Calkauskas, H. and Olsson, A.G. (1997) Antioxidant state and mortality from coronary heart disease in Lithuanian and Swedish men: concomitant cross sectional study of men aged 50. *British Medical Journal* 314, 629–653.

Kritchevsky, D. (1980) Pharmacology of probucol. In: Noseda, G., Lewis, B. and Paoletti, R. (eds) *Diet and Drugs in Atherosclerosis*. Raven Press, New York, pp. 143–149.

Kritchevsky, D. and Tepper, S.A. (1964) Autoxidation of methyl linoleate: effect of sex hormones and of nicotinic acid and related compounds. *Proceedings of the Society for Experimental Biology and Medicine* 115, 841–843.

Kritchevsky, D., Marcucci, A.M., Sallata, P. and Tepper, S.A. (1969) Comparison of amorphous and crystalline cholesterol in the establishment of atherosclerosis in rabbits. *Medicina Experimentalis* 19, 185–193.

Kritchevsky, D., Kim, H.K. and Tepper, S.A. (1971) Influence of 4-4′-(Isopropylidenedithio)bis(2,6-di-t-butylphenol) (DH-581) on experimental atherosclerosis in rabbits. *Proceedings of the Society for Experimental Biology and Medicine* 136, 1216–1221.

Kritchevsky, D., Tepper, S.A., Williams, D.E. and Story, J.A. (1977) Experimental atherosclerosis in rabbits fed cholesterol-free diets. 7. Interaction of animal or vegetable protein with fiber. *Atherosclerosis* 26, 397–403.

Kritchevsky, S.B., Shimakawa, T., Tell, G.S., Dennis, B., Carpenter, M., Eckfeldt, J.H., Preacher-Ryan, H. and Heiss, G. (1995) Dietary antioxidants and carotid artery wall thickness. The ARIC Study. *Circulation* 92, 2142–2150.

Kushi, L.H., Folsom, A.R., Prineas, R.J., Mink, P.J., Wu, Y. and Bostick, R.M. (1996) Dietary oxidant vitamins and death from coronary heart disease in post-menopausal women. *New England Journal of Medicine* 334, 1156–1162.

Morris, D.L., Kritchevsky, S.B. and Davis, C.E. (1994) Serum carotenoids and coronary heart disease. The Lipid Research Clinics Coronary Primary Prevention Trial and follow-up study. *Journal of the American Medical Association* 272, 1439–1441.

Moses, C., Rhodes, G.L. and Levinson, J.P. (1952) The effect of alpha tocopherol on experimental atherosclerosis. *Angiology* 3, 397–398.

Omenn, G.S., Goodman, G.E., Thornquist, M.D., Balmes, J., Cullen, M.R., Glass, A., Keogh, J.P., Meyskens, F.L. Jr, Valanis, B., Williams, J.H. Jr, Barnhart, S. and Hammar, S. (1996) Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *New England Journal of Medicine* 334, 1150–1155.

Parthasarathy, S., Young, S.G., Witztum, J.L., Pittman, R.C. and Steinberg, D. (1986) Probucol inhibits oxidative modification of low density lipoproteins. *Journal of Clinical Investigation* 77, 641–644.

Peng, S.-K., Taylor, C.B., Tham, P., Werthessen, N.T. and Mikkelson, B. (1978) Effect of auto-oxidation products from cholesterol on aortic smooth muscle cells. *Archives of Pathology and Laboratory Medicine* 102, 57–61.

Peng, S.-K., Tham, P., Taylor, C.B. and Mikkelson, B. (1979) Cytotoxicity of oxidation derivatives of cholesterol on cultured aortic smooth muscle cells and their effect on cholesterol biosynthesis. *American Journal of Clinical Nutrition* 32, 1033–1042.

Peng, S.-K., Phillips, G.A., Xia, G.Z. and Morin, R.J. (1987) Transport of cholesterol autoxidation products by rabbit lipoproteins. *Atherosclerosis* 64, 1–6.

Peng, S.-K., Hu, B. and Morin, R.J. (1991) Angiotoxicity and atherogenicity of cholesterol oxides. *Journal of Clinical Laboratory Analysis* 5, 144–152.

Pomerantz, K.B., Nicholson, A.C. and Hajjar, D.P. (1995) Signal transduction in atherosclerosis: second messengers and regulation of cellular cholesterol trafficking. *Advances in Experimental Medicine and Biology* 369, 49–64.

Rapola, J.M., Virtamo, J., Haukka, J.K., Heinonen, U.P., Albanes, D., Taylor, P.R. and Huttinen, J.K. (1996) Effect of vitamin E and beta carotene on the incidence of angina pectoris. A randomized, double-blind controlled trial. *Journal of the American Medical Association* 275, 693–698.

Rapola, J.M., Virtamo, J., Ripatti, S., Huttunen, J.K., Albanes, D., Taylor, P.R. and Heinonen, O.P. (1997) Randomised trial of α-tocopherol and β-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. *Lancet* 349, 1715–1720.

Retsky, K.L., Freeman, M.W. and Frei, B. (1993) Ascorbic acid oxidation product(s) protect human low density lipoprotein against atherogenic modification. Anti- rather than prooxidant activity of vitamin C in the presence of transition metal ions. *Journal of Biological Chemistry* 268, 1304–1309.

Riemersma, R.A., Wood, D.A., Macintyre, C.C.A., Elton, R.A., Gey, K.F. and Oliver, M.F. (1991) Risk of angina pectoris and plasma concentrations of vitamins A, C, E and carotene. *Lancet* 337, 1–5.

Rimm, E.B., Stampfer, M.J., Ascherio, A., Giovannucci, E., Colditz, G.A. and Willett, W.C. (1993) Vitamin E consumption and the risk of coronary heart disease in men. *New England Journal of Medicine* 328, 1450–1456.

Rimm, E.B., Katan, M.B., Ascherio, A., Stampfer, M.J. and Willett, W.C. (1996) Relation between intake of flavonoids and risk of coronary disease in male health professionals. *Archives of Internal Medicine* 125, 384–389.

Rosenfeld, M.E., Khoo, J.C., Miller, E., Parthasarathy, S., Palinski, W. and Witztum, J.L. (1991) Macrophage-derived foam cells freshly isolated from rabbit atherosclerotic lesions degrade modified lipoproteins, promote oxidation of low-density lipoproteins, and contain oxidation-specific lipid–protein adducts. *Journal of Clinical Investigation* 87, 90–99.

Ross, R. (1986) The pathogenesis of atherosclerosis – an update. *New England Journal of Medicine* 314, 488–500.

Shwaery, G.T., Vita, J.A. and Keaney, J.F. Jr (1997) Antioxidant protection of LDL by physiological concentrations of 17β-estradiol. Requirement for estradiol modification. *Circulation* 95, 1378–1385.

Singh, R.B., Niag, A.M., Ghosh, S., Agarwal, P., Ahmad, S., Begum, R., Onouchi, Z. and Kummerow, F.A. (1995) Randomized controlled trial of antioxidant vitamins and cardioprotective diet on hyperlipidemia, oxidative stress and development of experimental atherscelerosis: the diet and antioxidant trial on atherosclerosis (DATA). *Cardiovascular Drugs and Therapy* 9, 763–771.

Singh, R.B., Niaz, M.A., Rastogi, S.S. and Rastogi, S. (1996) Usefulness of antioxidant vitamins in suspected acute myocardial infarction (The Indian Experiment of Infarct Survival-3). *American Journal of Cardiology* 77, 232–236.

Slattery, M.L., Jacobs, D.R. Jr, Dyer, A., Benson, J., Hilmer, J.E. and Caan, B.J. (1995) Dietary antioxidants and plasma lipids: the CARDIA Study. *Journal of the American College of Nutrition* 14, 635–642.

Smith, L.L. and vanLier, J.E. (1970) Sterol metabolism IX. 26-hydroxycholesterol levels in the human aorta. *Atherosclerosis* 12, 1–4.

Smith, T.L. and Kummerow, F.A. (1989) Effect of dietary vitamin E on plasma lipids and atherogenesis in restricted ovulator chickens. *Atherosclerosis* 75, 105–109.

Stampfer, M.J., Hennekens, C.H., Manson, J.E., Colditz, G.A., Rosner, B. and Willett, W.C. (1993) Vitamin E consumption and the risk of coronary disease in women. *New England Journal of Medicine* 328, 1444–1449.

Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C. and Witztum, J.L. (1989) Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *New England Journal of Medicine* 320, 915–924.

Stephens, N.G., Parsons, A., Schofield, P.M., Kelly, F., Cheeseman, K., Mitchison, M.J. and Brown, J. (1996) Randomised controlled trial of vitamin E in patients with coronary diease: Cambridge Heart Antioxidant Study. *Lancet* 347, 781–786.

Street, D.A., Comstock, G.W., Salkeld, R.M., Schüep, W. and Klag, M.J. (1994) Serum antioxidants and myocardial infarction are low levels of carotenoids and alpha tocopherol risk factors for myocardial infarction? *Circulation* 90, 1154–1161.

Tedeschi, R.E., Taylor, H.L. and Martz, B.L. (1980) Clinical experience of the safety and cholesterol-lowering action of probucol. In: Noseda, G., Lewis, B. and Paoletti, R. (eds) *Diet and Drugs in Atherosclerosis*. Raven Press, New York, pp. 199–207.

Verlangieri, A.J. and Bush, M.J. (1992) Effects of d-α-tocopherol supplementation on experimentally induced primate atherosclerosis. *Journal of the American College of Nutrition* 11, 131–138.

Vogel, J. (1847) *The Pathological Anatomy of the Human Body*. Lea and Blanchard, Philadelphia, Pennsylvania.

White, C.R., Darley-Usmar, V. and Oparil, S. (1997) Gender and cardiovascular disease. Recent insights. *Trends in Cardiovascular Medicine* 7, 94–100.

Wilson, R.B., Middleton, C.C. and Sun, G.Y. (1978) Vitamin E, antioxidants and lipid peroxidation in experimental atherosclerosis of rabbits. *Journal of Nutrition* 108, 1858–1867.