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Cholesterol standardized plasma vitamin E levels are reduced in patients with severe angina pectoris

GORDON FERNS*, JULIE WILLIAMS†, LOUISE FORSTER†, SAMANTHA TULL†, BRYAN STARKEY* AND ANTHONY GERSHLICK‡ (ON BEHALF OF THE COVERT TRIALISTS)

**Department of Clinical Biochemistry, The Royal Surrey County Hospital,Guildford, Surrey and Departments of †Chemical Pathology and ‡Cardiology, Glenfield General Hospital, Leicester, UK*

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Summary. Vitamin E, the major lipid soluble plasma antioxidant, has been reported to be reduced in patients with coronary atherosclerosis. We have measured the levels of plasma α-tocopherol (the predominant form of plasma vitamin E) in 128 patients with different reported degrees of angina.

Patients with mild to moderate angina (grades I or II (CSS score)) ($n = 64$), and patients with severe angina (grades III and IV) ($n = 64$) were recruited from Cardiology Clinics in the U.K. Healthy controls ($n = 33$) and patients with hyperlipidaemia ($n = 28$) were also recruited.

The groups of patients with angina did not differ significantly for mean age (58 ± 1.0 years *vs.* 59 ± 1.0 years, respectively); sex distribution (the M:F ratio was 48 : 16 and 46 : 18 for the respective groups); or prevalence of smoking (12% *vs.* 9%), or hypertension (19% *vs.* 33%). Total cholesterol levels were higher in the group with severe angina (5.9 ± 0.16 mmol/l *vs.* 5.3 ± 0.13 mmol/l $P < 0.05$). Absolute levels of plasma vitamin E were not significantly different between the angina subgroups (12.9 ± 0.40 mg/l for the mild-moderate angina group *vs.* 12.5 ± 0.51 mg/l for the severely affected group), but were positively correlated with plasma cholesterol concentrations in each case ($P < 0.001$). The ratio between plasma vitamin E: total cholesterol was significantly lower in the patients with severe angina (mean 2.20 ± 0.09 mg/mmol) *vs.* a mean value of 2.46 ± 0.08 mg/m mol in the mildly affected group ($P < 0.05$). The plasma vitamin E: total cholesterol ratio in patients with severe angina was also significantly lower ($P < 0.05$) compared to either healthy controls with comparable total cholesterol levels ($n = 33$), or hypercholesterolaemic subjects ($n = 28$) without symptomatic coronary disease (mean ratios were 2.69 ± 0.40 mg/mmol and 2.74 ± 0.68 mg/mmol, respectively).

Vitamin E has previously been demonstrated to protect endothelial function in the presence of hypercholesterolaemia, possibly by preserving nitric oxide

Correspondence: Professor G. A. A. Ferns, Centre for Clinical Science & Measurement, School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH, UK. Fax: +44 (0)1483 464072; E-mail: g.ferns@surrey.ac.uk

bio-activity. It also inhibits LDL oxidation. Hence, a high plasma vitamin E: total cholesterol ratio may be associated with an amelioration of angina.

Keywords: Vitamin E, angina, clinical

Vitamin E is a potent lipid-soluble plasma antioxidant (Burton *et al*. 1983). Subjects who take supplements of vitamin E have been shown to be at lower risk of coronary heart disease(Rimm *et al*. 1993; Stampfer *et al*. 1993). Vitamin E supplementation has also been shown to reduce morbidity in patients with established coronary hear disease (Stephens *et al*. 1996). The mechanisms by which vitamin E exerts its effects are unclear, but we and colleagues have shown it to inhibit platelet aggregation (Steiner 1983; Kakishita *et al*. 1990; Violo *et al*. 1990; Salonen *et al*. 1991; Williams *et al*. 1997) and adhesion (Jandak *et al*. 1989). Vitamin E is lipophilic and has been shown to inhibit the oxidative modification of low density lipoprotein (LDL), a process thought to be of crucial importance in atherogenesis (Steinberg *et al*. 1989). We and colleagues have also previously demonstrated that α-tocopherol (the biologically most potent isomer of vitamin E) has important direct effects on vascular endothelial cells, monocytes and smooth muscle cells (Steiner 1981; Boscoboinik *et al*. 1991; Faruqi *et al*. 1994; Stewart-Lee *et al*. 1994; Konneh *et al*. 1995; Devaraj *et al*. 1996; Calzada *et al*. 1997; Erl *et al*. 1997; Williams *et al*. 1997). These effects include the preservation of endothelium-dependent relaxation (Stewart-Lee *et al*. 1994), inhibition of monocyte adhesion (Devaraj *et al*. 1996; Erl *et al*. 1997) and cytokine expression (Devaraj *et al*. 1996), and suppression of smooth muscle cell proliferation (Boscoboinik *et al*. 1991; Konneh *et al*. 1995). In the present study we have measured the plasma levels of α-tocopherol in patients with established coronary artery disease but with different degrees of angina; in healthy controls and in patients with asymptomatic hypercholesterolaemia.

Materials and methods

Subjects

Patients with angiographically defined coronary heart disease were recruited from the Cardiology Clinics at Glenfield General Hospital, Leicester, Broad Green Hospital, Liverpool and Papworth Hospital, Cambridge. Each patient had > 70% stenosis of one or more epicardial coronary arteries and subsequently underwent coronary angioplasty as part of the Coronary Vitamin E Restenosis Trial (COVERT). Angina grade (CSS class, I (mild) to IV (severe)) was assessed by direct questioning during a clinical examination by a Consultant, or Specialist Cardiologist. Hypercholesterolaemic patients ($n = 28$) were recruited from those newly referred to the Lipid Clinic at Glenfield General Hospital, Leicester, or the Royal Surrey County Hospital, Guildford, with a diagnosis of primary hypercholesterolaemia (serum cholesterol > 5.7 mmol/l but < 10.0 mmol/l; and serum triglycerides < 3.3 mmol/l). Exclusion criteria included: a positive recent smoking habit, diabetes mellitus or hypothyroidism. Healthy controls ($n = 33$) were recruited from Hospital Trust and University staff. Ethical approval was obtained before the commencement of the study and conformed with the principles outlined in the Declaration of Helsinki.

Blood sampling and lipid profiles

Plasma was obtained from fasting blood samples collected into lithium-heparin tubes (1.5 i.u. heparin/ml) following a 12-h overnight fast and centrifuged at 1500 g for 10 min at 4 °C. Measurements of plasma total-and HDL-cholesterol, and triglycerides were made using a Kodak Ektachem 700XR Analyser C series (Eastman Kodak Company, Rochester, USA).

Plasma antioxidant vitamin levels

Plasma samples were obtained from lithium heparin blood samples as described above, and stored at −70 °C in the dark until analysis. Plasma α-tocopherol was determined by HPLC using a modification of the method of Bieri *et al*. (1979). Briefly, 200 μl internal standard (10 μg/ml d-tocopherol in isopropyl alcohol) was added to 200 μl serum and vortex mixed. Aqueous ammonium sulphate (3.9 M) was added (200 μl) and the solution was again vortex mixed. After centrifugation (1000 g for 5 min), 50 μl of supernatant was for analysis using a Prodigy 5 μM ODS2 (50 × 4.6 mm) column (Phenomenex Ltd, Macclesfield, Cheshire, U.K.) with methanol as the mobile phase, and detection at 294 nm. At a flow rate of 2 ml/minute, the retention times for internal standard and α-tocopherol were 3.3 and 4.2 min, respectively. α-tocopherol standard and quality control material (BioRad Laboratories Ltd, Hemel Hempstead, U.K.) were used to calibrate the assay and ensure acceptable batch to batch reproducibility. Inter-assay precision data gave coefficients of variation of 5.1% and 3.9% at α-tocopherol concentrations of 3.1 and 10.8 μg/l, respectively.

Statistical analysis

Results were expressed as mean ± SEM. Significance was assessed by using Mann–Whitney and paired *t*-tests for continuous data, and chi-square, or Fisher's exact tests for categorical data; the significance of the relationship between continuous variables was assessed by Pearson's correlation.

Results

Patient characteristics

A total of 128 subjects with coronary disease were recruited into the study; 64 with mild-moderate angina (grade I and II) (male: female 48: 16); mean age 58 ± 1.04 years, and 64 with severe angina (grade III and IV) (male: female ratio 46: 18); mean age 59. ± 1.0 years. Their clinical characteristics are summarized in Table 1. Of the 128 subjects, detailed angiographic data were available for 126, of whom 103 had single vessel disease, and 23 had multiple vessel disease. The clinical characteristics of these subgroups are presented in Table 2. Twenty-eight hypercholesterolaemic subjects and 33 controls were also recruited. Their characteristics are also presented in Table 1. Each group comprised subjects of a similar mean age ($P > 0.05$). There was no significant difference in the male: female ratio between the two subgroups with CHD, although they had a larger proportion of males than either the control group, or

Table 1. Characteristics of patients with coronary heart disease with different degrees of reported angina, subjects with hypercholesterolaemia and healthy controls

	Mild–moderate angina	Severe angina	Controls	Hyper-cholesterolaemia
n	64	64	33	28
Mean age (years)	58 ± 1.04	59 ± 1.0	56.1 ± 1.6	55.2 ± 2.1
M:F ratio	48 : 16	46 : 18	22 : 11	15 : 13
Current smokers (%)	12	9	0	0
Hypertensive (%)	19	33	0	0
Receiving drug therapy β-blocker (%)	55	59	0††	0††
Lipid lowering drugs (%)	22	30	0‡	0†
Lipoprotein profile				
Mean total plasma cholesterol (mmol/l)	5.3 ± 0.13	5.9 ± 0.16**	5.9 ± 0.4	7.1 ± 0.15*
Mean plasma HDL cholesterol (mmol/l)	1.22 ± 0.05	1.15 ± 0.05	1.35 ± 0.6	1.29 ± 0.05
Mean total plasma triglycerides (mmol/l)	2.10 ± 0.12	2.16 ± 0.20	2.20 ± 0.30	1.98 ± 0.15
Plasma α-tocopherol concentration (mg/l)	12.9 ± 0.40	12.5 ± 0.51	15.1 ± 1.7	19.4 ± 0.95*
Plasma α-tocopherol/plasma total cholesterol (mg/mmol)	2.46 ± 0.08	2.20 ± 0.09**	2.69 ± 0.07	2.74 ± 0.13

Values are mean ± sem; **$P < 0.05$ compared to patients with mild angina by *t*-test; *$P < 0.01$ compared to controls by *t*-test; ‡ $P < 0.01$ compared to patients with mild angina and $P < 0.001$ compared to patients with severe angina by Fisher's exact test; † $P < 0.01$ compared to patients with mild angina and $P < 0.002$ compared to patients with severe angina by Fisher's exact test; †† $P < 0.0001$ compared to patients with mild angina and compared to patients with severe angina by Fisher's exact test.

Table 2. Characteristics of patients with coronary heart disease with different degrees of angiographically defined disease

	Single vessel disease	Multiple vessel disease
n	103	23
mean age (years)	59.7 ± 0.9	58.4 ± 1.6
M:F ratio	77 : 26	15 : 8
Current smokers (%)	11	9
Hypertensives (%)	26	30
Lipoprotein profile		
Mean total plasma cholesterol (mmol/l)	5.7 ± 0.13	5.9 ± 0.22
Mean plasma HDL cholesterol (mmol/l)	1.19 ± 0.04	1.18 ± 0.06
Mean total plasma triglycerides (mmol/l)	2.14 ± 0.12	2.17 ± 0.23
Plasma α-tocopherol concentration (mg/l)	13.3 ± 0.45	11.7 ± 0.55
Plasma α-tocopherol/plasma total cholesterol (mg/mmol)	2.3 ± 0.10	2.1 ± 0.14

Values are mean ± sem.

group of patients with hypercholesterolaemia. A similar proportion of smokers were in both CHD subgroups. The control and hyperlipidaemic subjects were all non-smokers, and neither of these groups contained hypertensive subjects.

Plasma lipid levels

The hypercholesterolaemic subjects had significantly higher plasma cholesterol levels than the other groups. Neither plasma triglycerides, nor HDL-cholesterol levels differed significantly among the groups. The subgroup of the patients with established CHD with severe angina had higher plasma levels of total cholesterol than those with mild disease (5.9 ± 0.16 *vs.* 5.3 ± 0.13 mmol/l, respectively; $P < 0.05$). There was no significant difference in the proportion of patients with different degrees of angina who were on lipid lowering treatment (22% *vs.* 30%; $P > 0.05$), nor on β-blockers (55% *vs.* 59%; $P > 0.05$). And subjects with different degrees of coronary stenosis defined by angiography, did not differ with regard to levels plasma cholesterol.

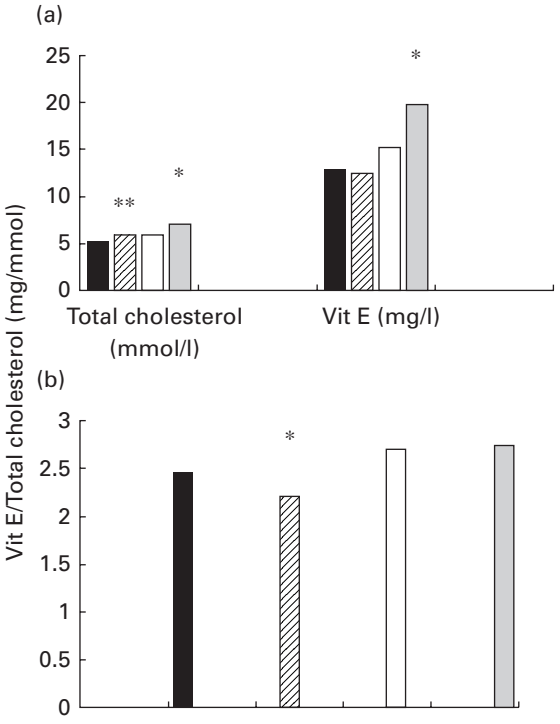

Figure 1. a) Plasma vitamin E and total cholesterol levels, and (b) Plasma vitamin E: total cholesterol ratio in patients with different degrees of angina, hypercholesterolaemic patients and healthy controls. ■ Grade I/II; ▨ Grade III/IV; □ Controls; ▩ Hypercholesterolaemics (HC) $^{**}P < 0.05$ compared to patients with mild-moderate angina by *t*-test; $^{*}P < 0.01$ compared to control subjects, and patients with mild-moderate angina.

Plasma vitamin E levels

Patients with hypercholesterolaemia had significantly ($P < 0.01$) higher levels of plasma α-tocopherol than the other groups (Figure 1 and Table 1). The subgroups of patients with CHD had lower levels of plasma α-tocopherol than control subjects, although this failed to reach statistical significance ($P > 0.05$). Nor did the value of plasma α-tocopherol differ significantly between these subgroups. However when standardized for plasma total cholesterol, mean plasma α-tocopherol/total cholesterol values were significantly lower in the group of subjects with severe angina, whilst the value in the hypercholesterolaemic subjects was no longer significantly different from the controls ($P > 0.05$).

There was a positive association between concentrations of plasma cholesterol and α-tocopherol in both subgroups of patients with angina ($P < 0.01$; Figure 2).

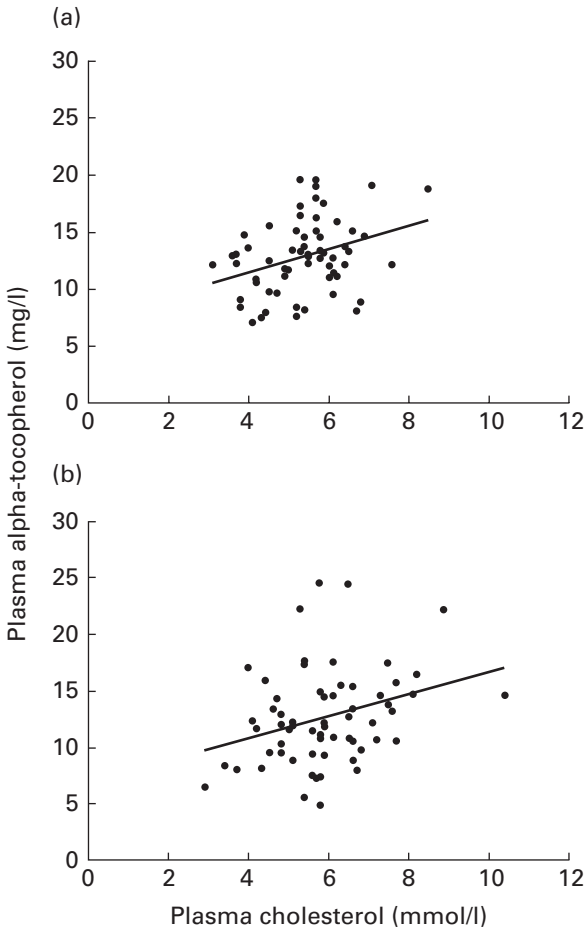

Figure 2. Relationship between plasma cholesterol levels and plasma α-tocopherol levels in patients with (a) mild-moderate angina ($R^2 = 0.112$) or (b) severe angina ($R^2 = 0.0946$). There was a weak, though significant positive correlation ($P < 0.001$) between these two parameters in each case.

Discussion

The lipid oxidation hypothesis of Steinberg *et al.* (1989) proposes that oxidative modification of LDL is a crucial step in the early phase of atherogenesis. It follows that factors that prevent LDL oxidation should also protect against the development of atherosclerosis. There is growing epidemiological evidence supporting this hypothesis, particularly relating to the benefits of high plasma concentrations of vitamin E. Gey *et al.* (1993) have reported an inverse relationship between mortality from ischaemic heart disease and concentrations of plasma vitamin E. Riemersma *et al.* (1991) have also reported that plasma vitamin E concentrations are inversely and independently related to the risk of angina in a Scottish population. More recently Duthie *et al.* (1994) were unable to confirm this, but did report higher indices of lipid peroxidation among patients with angina. Kim *et al.* (1996) have also reported significantly lower levels of plasma vitamin E in Korean patients with angiographically defined coronary artery disease. However, Karmansky *et al.* (1996) did not find differences in the plasma levels of products of lipid peroxidation, or plasma vitamin E in men with stable angina. Nor did they find differences in lag-time, maximal rate of oxidation, and total amount of conjugated dienes in their assessment of LDL oxidisability, in LDL isolated from groups of individuals with angiographically defined coronary atherosclerosis *vs.* controls. Nevertheless this latter study was in a small number of subjects with stable angina. Miwa *et al.* (1996a) have reported that plasma vitamin E levels in the LDL fraction were significantly lower in normocholesterolaemic Japanese patients with active variant angina than in subjects without coronary spasm, including patients with $>75\%$ stenosis, but with stable angina, implying an association between vitamin E deficiency and coronary artery spasm. They also report that the LDL isolated from these patients with variant angina was more susceptible to copper-induced oxidation *in vitro* (Miwa *et al.* 1996b).

Data from intervention trials and longitudinal studies also suggest that vitamin E is protective against atherogenesis (Rimm *et al.* 1993; Stampfer *et al.* 1993; Rapola *et al.* 1996; Stephens *et al.* 1996). Benefits of vitamin E were only observed in healthy individuals taking additional dietary supplements, but not in those subjects with a high unsupplemented dietary vitamin E consumption (Rimm *et al.* 1993; Stampfer *et al.* 1993). In the CHAOS trial, vitamin E supplements at a dose between 400 and 800 i.u. per day reduced the risk of nonfatal myocardial infarction in patients with existing disease (Stephens *et al.* 1996).Dietary supplementation with alpha tocopherol was also found to be associated with a small decrease in the incidence of angina pectoris ($P<0.05$) in Finnish male smokers who were without a previous history of known coronary heart disease (Rapola *et al.* 1996).

In this present study we have found that patients with severe angina (Grade III and IV) have significantly lower levels of cholesterol-standardized plasma vitamin E than patients with mild angina (Grades I and II), or individuals without overt coronary disease. However we did not find any significant differences in cholesterol-standardized vitamin E levels in relation to extent of angiographically defined disease. These data suggest that vitamin E may be associated with an amelioration in the symptoms of angina, possibly by preserving endothelial function and maintaining coronary blood flow. Exposure to oxidative modified LDL has been reported to increase coronary responses to vasoconstrictors, and to impair responses to vasodilators (Plane *et al.* 1992; Murohara *et al.* 1994; Cox & Cohen 1996). Deficiency of vitamin E, a major plasma antioxidant, may be related to the occurrence of coronary artery spasm.

However there are alternative interpretations of the data. It is possible that subjects with severe angina have social or dietary habits that exacerbate their angina and also deplete their plasma antioxidants. For example they may consume more tobacco, or have diets lower in fresh fruit and vegetables. These details were not obtained for the subjects in the current study, and therefore cannot be excluded. It is also possible that recurrent attacks of angina and generalized poor tissue perfusion, which may be more profound in the patients with severe angina, result in a greater utilization of protective antioxidants. High levels of plasma vitamin E may also promote the angiogenic responses necessary for the formation of collateral vessels in the myocardium.

Conclusion

Plasma concentrations of standardized vitamin E are low in subjects with severe angina. It is uncertain whether this relationship is due to cause or consequence. This question may possibly be addressed by examining the effects of vitamin E supplementation on angina severity.

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References

Bieri J.G., Tolliver T.J. & Catignani G.L. (1979) Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *Am. J. Clin. Nutr.* **32,** 2143–2149.

Boscoboinik D., Szewczyk A. & Azzi A. (1991) Alpha-tocopherol regulates vascular smooth muscle cell proliferation and protein kinase C activity. *Arch. Biochem. Biophys.* **286,** 264–269.

Burton G.W., Joyce A. & Ingold K.U. (1983) Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? *Arch. Biochem. Biophys.* **221,** 281–290.

Calzada C., Bruckdorfer K.R. & Rice-Evans C.A. (1997) The influence of antioxidant nutrients on platelet function in healthy volunteers. *Atherosclerosis.* **128,** 97–105.

Cox D.A. & Cohen M.L. (1996) Selective enhancement of 5-hydroxytryptamine-induced contraction of porcine coronary artery by oxidized low-density lipoprotein. *J. Pharmacol. Exp. Ther.* **276,** 1095–1103.

Devaraj S., Li D. & Jialal I. (1996) The effects of alpha tocopherol supplementation on monocyte function. Decreased lipid oxidation, interleukin 1 beta secretion, and monocyte adhesion to endothelium. *J. Clin. Invest.* **98,** 756–763.

Duthie G.G., Beattie J.A., Arthur J.R., Franklin M., Morrice P.C. & James W.P. (1994) Blood antioxidants and indices of lipid peroxidation in subjects with angina pectoris. *Nutrition* **10,** 313–316.

Erl W., Weber C., Wardemann C. & Weber P.C. (1997) α-Tocopheryl succinate inhibits monocytic cell adhesion to endothelial cells by suppressing NF-kappa B mobilization. *Am. J. Physiol.* **A273,** H634–H640.

Faruqi R., De La Motte C. & Dicorleto P.E. (1994) α-tocopherol inhibits agonist-induced monocytic cell adhesion to cultured human endothelial cells. *J. Clin. Invest.* **94,** 592–600.

Gey K.F., Moser U.K., Jordan P., Stahelin H.B., Eichholzer M. & Ludin E. (1993) Increased risk of cardiovascular disease at suboptimal plasma concentrations of essential antioxidants: an epidemiological update with special attention to carotene and vitamin C. *Am. J. Clin. Nutr* **57,** 787S–797S.

Jandak J., Steiner M. & Richardson P.D. (1989) Alpha-tocopherol an effective inhibitor of platelet adhesion. *Blood* **73,** 141–149.

Kakishita E., Suehiro A., Oura Y. & Nagaik K. (1990) Inhibitory effect of vitamin E on spontaneous aggregation in whole blood. *Thromb. Res.* **60,** 489–499.

Karmansky I., Shnaider H., Palant A. & Gruener N. (1996) Plasma lipid oxidation and susceptibility of low-density lipoproteins to oxidation in male patients with stable coronary artery disease. *Clin. Biochem.* **29,** 573–579.

Kim S.Y., Lee-Kim Y.C., Kim M.K. *et al.* (1996) Serum levels of antioxidant vitamins in relation to coronary artery disease: a case control study of Koreans. *Biomed. Environ. Sci.* **9,** 229–235.

Konneh M., Rutherford C., Li S.-R., Aanggard E.E. & Ferns G.A.A. (1995) Vitamin E inhibits the intimal response to balloon catheter injury in the carotid artery of the cholesterol-fed rat. *Atherosclerosis* **113,** 29–39.

Miwa K., Miyagi Y., Igawa A., Nakagawa K. & Inoue H. (1996a) Vitamin E deficiency in variant angina. *Circulation* **94,** 14–18.

Miwa K., Miyagi Y. & Fujita M. (1996b) Susceptibility of plasma low density lipoprotein to cupric ion-induced peroxidation in patients with variant angina. *J. Am. Coll. Cardiol.* **28,** 1637–1638.

Murohara T., Kugiyama K., Ohgushi M., Sugiyama S., Ohta Y. & Yasue H. (1994) LPC in oxidized LDL elicits vasocontraction and inhibits endothelium- dependent relaxation. *Am. J. Physiol.* **267,** H2441–H2449.

Plane F., Bruckdorfer K.R., Kerr P., Steuer A. & Jacobs M. (1992) Oxidative modification of low-density lipoproteins and the inhibition of relaxations mediated by endothelium-derived nitric oxide in rabbit aorta. *Br. J. Pharmacol.* **105,** 216–222.

Rapola J.M., Virtamo J., Haukka J.K. *et al.* (1996) Effect of vitamin E and beta carotene on the incidence of angina pectoris. A randomized, double-blind, controlled trial. *J. A M. A.* **275,** 693–698.

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

Rimm E.B., Stampfer M.J., Ascherio A., Giovanniscu E., Colditz G.A. & Willett W.C. (1993) Vitamin E consumption and the risk of coronary heart disease in men. *N. Engl. J. Med.* **328,** 1450–1456.

Salonen J.T., Salonen R., Seppanen K. *et al.* (1991) Effects of antioxidant supplementation on platelet function in a randomised pair-matched, placebo-controlled, double-blind trial with men with low antioxidant status. *Am. J. Clin. Nutr.* **53,** 1222–1229.

Stampfer M.J., Hennekens C.H., Manson J.E., Colditz G.A., Rosner B. & Willett W.C. (1993) Vitamin E consumption and the risk of coronary heart disease in women. *N Engl. J. Med.* **328,** 1444–1449.

Steinberg D., Parathasarathy S., Carew T.E., Khoo J. & Witztum J.L. (1989) Beyond cholesterol. Modifications of low density lipoprotein that increases atherogenesis. *N. Engl. J. Med.* **20,** 915–924.

Steiner M. (1983) Effects of alpha-tocopherol administration on platelet function in man. *Thomb. Haemostasis* **49,** 73–77.

Steiner M. (1981) Vitamin E changes membrane fluidity of human platelets. *Biochim. Biophys. Acta* **640,** 100–105.

Stephens N.G., Parsons A., Schofield P.M. *et al.* (1996) Randomised controlled trial of vitamin E in patients with coronary disease. Cambridge Heart Antioxidant Study (CHAOS). *Lancet* **347,** 781–786.

Stewart-Lee A.L., Forster L.A., Nourooz-Zadeh J., Ferns G.A.A. & Anggard E.E. (1994) Vitamin E protects against impairment of endothelium-mediated relaxations in cholesterol-fed rabbit. *Arterioscler.* **14,** 494–499.

Violo F., Practico D., Ghiselli A. *et al.* (1990) Inhibition of cyclooxygenase independent platelet aggregation by low vitamin E concentration. *Atherosclerosis* **82,** 247–252.

Williams J., Forster L., Tull S., Wong M., Bevan R. & Ferns G. (1997) Dietary vitamin E supplementation inhibits thrombin-induced platelet aggregation, but not monocyte adhesiveness in patients with hypercholesterolaemia. *Int. J. Exp. Path.* **78,** 259–266.