Tissue Distribution of α-Tocopherol Following Dietary Supplementation in the Rat: Effects of Concomitant Cholesterol Feeding (43935)

MATTHEW K. KONNEH,*,[1] CLAIRE RUTHERFORD,* ERIK ANGGARD,* AND GORDON A. A. FERNS†

William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ, United Kingdom, and Department of Chemical Pathology,† Glenfield General Hospital, University of Leicester, Groby Rd, Leicester LE3 9QP, United Kingdom*

Abstract. **Vitamin E is a potent, naturally occurring, lipid-soluble antioxidant, which is reported to be protective against several disease processes, including coronary atherosclerosis. We have measured the α-tocopherol content of the aorta, liver, skeletal muscle, and kidney of rats fed one of the following diets for 10 weeks: a normal control chow diet (i); or the same diet containing 1% cholesterol (ii); 0.5% vitamin E (iii); or 1% cholesterol plus 0.5% vitamin E (iv). The α-tocopherol content of serum and tissue extracts was measured by HPLC using γ-tocopherol as an internal standard. Tissue and serum cholesterol content was measured using a cholesterol oxidase enzyme reagent kit. In all animals receiving the 1% cholesterol diet, serum cholesterol levels increased significantly ($P < 0.005$). By the 10th week, mean serum α-tocopherol levels rose significantly in both groups of animals receiving dietary vitamin E supplements ($P < 0.0001$) compared with their respective control group. This was accompanied by a significant increase in the absolute α-tocopherol content of liver (8- to 9-fold) and aorta (3- to 4-fold). The α-tocopherol content of renal and skeletal muscle tissue was raised 1- to 2-fold in both groups of rats on vitamin E supplements, however the increase attained significance only for the renal tissue. The aortic tissue α-tocopherol/cholesterol ratio was 4-fold higher in the rats receiving concomitant 1% cholesterol plus 0.5% vitamin E compared with animals receiving 1% cholesterol alone ($P < 0.02$), and was 5-fold higher in the rats receiving 0.5% vitamin E compared with those receiving control chow ($P < 0.01$). These data suggest that dietary vitamin E supplementation results in a differential uptake of α-tocopherol, which may be dependent, in part, on selective lipoprotein particle accumulation.** [P.S.E.B.M. 1995, Vol 210]

Vitamin E is a collective term for eight naturally occurring isomers, comprising the α-, β-, γ- and δ-tocopherols, and the α-, β, γ- and δ-tocotrienols (1). The tocopherols and tocotrienols are all lipophilic antioxidants. Of the eight isomers of vitamin E, α-tocopherol has the highest biological potency (2), and is the predominant form of vitamin E in blood.

Vitamin E has no specific plasma transport protein. It is carried in blood within the plasma lipoprotein particles, particularly low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (3, 4). Vitamin E fulfills its role as an antioxidant, protecting cells from damage associated with oxidative stress, in synergy with other circulating, and cellular antioxidants, including vitamin C (5).

Vitamin E has a therapeutic potential as an antiatherogenic agent (6, 7) because it may inhibit several of the key processes in atherogenesis, including platelet deposition, monocyte adherence, platelet-derived growth factor expression, smooth muscle cell proliferation, and LDL oxidation (6, 8–12). We have recently demonstrated that vitamin E can also inhibit neointimal hyperplasia following balloon catheter injury in the rat (13), a model of percutaneous transluminal cor-

[1] To whom requests for reprints should be addressed at William Harvey Research Institute, Charterhouse Square, London EC1M 6BQ, United Kingdom.

Received March 21, 1995. [P.S.E.B.M. 1995, Vol 210]
Accepted June 20, 1995.

0037-9727/95/2105-0000$10.50/0

onary angioplasty, a procedure commonly used in the treatment of symptomatic coronary heart disease.

There have been several studies of the pharmacokinetics and tissue distribution of vitamin E following dietary supplementation in various animal models (14–18). Machlin and Gabriel (19) reported that several weeks of vitamin E supplementation are required to saturate tissues with α-tocopherol. No previous studies have investigated the effects of concomitant cholesterol feeding on α-tocopherol tissue accumulation. We were particularly interested in uptake by vascular tissue because of the proposed anti-atherogenic properties of vitamin E.

Material and Methods

Rat Colonies. Adult, male Wistar rats, 8–10 weeks old, were obtained as a gift from Glaxo (Greenford, United Kingdom) and were housed in the Biological Services Unit of St. Bartholomew's Hospital Medical College.

Dietary Groups. Rats were initially maintained on a commercial rat chow diet (Scientific Diet Services, Essex, United Kingdom). They were allocated to one of four dietary groups approximately 1 month after delivery, when they were approximately 400 g in weight. The dietary groups were: (i) control commercial chow containing 0.007% vitamin E (w/w), (ii) chow with 1% added cholesterol (Sigma, Dorset, United Kingdom), (iii) chow with 0.5% added vitamin E (as ± α-tocopherol acetate, Sigma), and (iv) chow with 1% cholesterol plus 0.5% vitamin E. Water was allowed *ad libitum*. The cholesterol-containing diets were prepared by spraying the standard chow with a solution of cholesterol dissolved in diethyl ether (20). The vitamin-enriched diets were similarly prepared by spraying the standard chow or cholesterol-containing diet with an ethereal solution of α-tocopherol (20). The prepared diets were dried overnight to ensure complete evaporation of the solvent.

Blood Sampling. Venous blood was collected by tail bleeds from each animal before the start of each experimental diet and at the time of sacrifice. Blood samples were separated by centrifuging at 1000*g* for 10 min at 25°C. The serum was then stored at −20°C until analysis.

Animal Killing. After 10 weeks of the experimental diet, rats were sedated with xylazine (Rompun, 4 mg/100 g body wt; Bayer, Suffolk, United Kingdom) and ketamine (Vetalar, 1 mg/100 g body wt; Parke-Davis Inc., Pontypool, United Kingdom). An abdominal incision was made and blood collected via a trochar inserted into the abdominal aortic bifurcation. The rats were sacrificed by exsanguination, and tissues collected immediately. Samples of liver, skeletal muscle (rectus abdominus), kidney, and thoracic aorta were dissected free of fascia and snap frozen in liquid nitrogen. Tissues were stored at −70°C until analysis.

Serum Vitamin E Analysis. Serum α-tocopherol levels were measured by HPLC as previously reported (20) using γ-tocopherol (Sigma) as an internal standard.

Tissue Vitamin E Analysis. Frozen tissues were cut and weighed (100–200 mg). Two milliliters dichloromethane (Rathburn Chemicals, Walkerburn, United Kingdom) was added and the tissue homogenized using an IKA T25 homogenizer (Labortechnik, Germany). One milliliter of distilled water and 1 ml ethanol, containing 500 ng γ-tocopherol, were added and the tubes vortex mixed. After centrifuging at 1000*g* for 5 min, the lower dicholoromethane layer was recovered and divided into two equal aliquots which were dried under nitrogen. One aliquot was reconstituted in acetonitrile and analyzed for α-tocopherol content by HPLC as described above. Vitamin E recovery experiments were performed by adding 0.5 μg of α-tocopherol to the tissue homogenate samples prior to organic extraction. The other aliquot was used for the analysis of tissue cholesterol content.

Tissue Cholesterol Analysis. The aliquots of tissue extract were reconstituted in 0.5 ml propan-2-ol (Rathburn Chemicals, Walkerburn, United Kingdom). Ten microliters of cholesterol standard or reconstituted tissue extract were placed into a glass tube, and 100 μl of ethanolic potassium hydroxide (2 ml of 12 *M* potassium hydroxide plus 48 ml absolute ethanol) was added. The tubes were capped, vortex mixed, and placed into a boiling water bath for 2 min. The tubes were cooled to 0°C, and 1 ml of cholesterol oxidase (0.25 U/ml; Boehringer-Mannheim, Lewes, United Kingdom) was added. The tubes were incubated for a further 15 min at 50°C and the reaction stopped by the addition of 0.9 ml ethanolic potassium hydroxide. Two milliliters of iso-octane (Rathburn) was then added to each tube, which was vortex mixed and centrifuged at 3000*g* for 10 min. The absorbance of the iso-octane layer was measured at 232 nm in a UV spectrophotometer, and cholesterol concentrations determined by reference to a standard curve (21).

Serum Cholesterol Analysis. Serum cholesterol concentrations were measured by the cholesterol-oxidase-peroxidase colorimetric method using a cholesterol C-system kit (Boehringer-Mannheim) on a Vitalab 100 autoanalyser (Vitalab Scientific Ltd., Sussex, United Kingdom) with Precipath U and Precinorm U (Boehringer-Mannheim) quality control material.

Statistics. Statistical analyses were performed on an IBM-compatible personal computer using INSTAT software (GraphPAD Software, San Diego, CA). Differences between means were analyzed by paired, or unpaired, Student *t* tests. Linear regression was used to assess the relationship between α-tocopherol and

cholesterol. A probability of <0.05 was considered significant.

Results

Effects of Experimental Diets on Body Weight, Serum α-Tocopherol, and Serum and Tissue Cholesterol Levels. All animals gained weight throughout the duration of the experimental period. Weight gain and final weights did not differ significantly between the groups ($P > 0.05$; Table I). After 10 weeks of the experimental diet, serum α-tocopherol levels were three-fold higher in the animals receiving vitamin E plus cholesterol compared to those fed on a cholesterol-only diet ($P < 0.001$). Serum α-tocopherol levels were 5-fold higher in animals fed the chow supplemented with 0.5% vitamin E alone compared with animals on the control chow ($P < 0.0001$).

In animals receiving the 1% cholesterol diets, serum cholesterol levels were significantly higher at the time of sacrifice compared with basal levels ($P < 0.005$; Table I), and were also significantly higher than in rats fed the control chow diet ($P < 0.05$). Serum α-tocopherol concentrations were directly related to serum cholesterol levels when all the experimental animal groups were pooled ($r = 0.48$, $P = 0.016$), and were particularly strongly correlated for the group of animals receiving dietary cholesterol plus vitamin E ($r = 0.97$, $P = 0.0012$). Both groups of animals receiving diets containing vitamin E had a significantly higher serum α-tocopherol/cholesterol ratio at the time of sacrifice compared with values prior to starting the experimental diets ($P < 0.05$; Table I).

Effects of Experimental Diets on the Tissue Distribution of α-Tocopherol. The recovery of α-tocopherol from each of the tissues investigated was approximately 90% (liver 96.4% ± 4.5%; aorta 92.3% ± 4.1%; kidney 94.7% ± 3.2%; and skeletal muscle 89.1% ± 4.8%).

Liver. Of the tissues studied, the liver contained the highest absolute levels of α-tocopherol for each experimental group. The α-tocopherol/cholesterol ratio was also highest for hepatic tissue, irrespective of whether animals received concomitant dietary cholesterol supplementation.

The hepatic α-tocopherol content was significantly higher in rats receiving dietary vitamin E alone compared with rats fed control chow ($P < 0.0001$), or cholesterol alone ($P < 0.05$) (Fig. 1a). Although the mean hepatic α-tocopherol levels were higher in animals receiving concomitant cholesterol (162 ± 36.2 μg/g) than in rats receiving vitamin E alone (114.9 ± 15.7 μg/g), this failed to reach statistical significance. The hepatic α-tocopherol/cholesterol ratio was 8-fold higher in the animals receiving vitamin E alone than in rats on the control chow diet ($P < 0.0001$) and 4-fold higher in the animals receiving vitamin E plus cholesterol versus those on cholesterol only ($P < 0.05$) (Fig. 1b). There was a significant positive association between hepatic α-tocopherol and hepatic cholesterol content ($r = 0.43$, $P = 0.026$; Fig. 2a).

Aorta. Aortic α-tocopherol content was significantly higher in both groups of animals receiving dietary vitamin E supplements compared to their respective control group ($P < 0.02$ in both cases; Fig. 1a). The absolute aortic α-tocopherol content was lower than hepatic levels but higher than those found in renal and skeletal muscle tissue (Fig. 1a). The aortic α-tocopherol/cholesterol ratio was approximately 4-fold higher in animals fed vitamin E plus cholesterol than in those receiving cholesterol alone ($P < 0.05$) (Fig. 1b), and 5-fold higher in animals fed vitamin E alone than in those on the control chow diet ($P < 0.05$). Aortic α-tocopherol content was positively related to aortic cholesterol content ($r = 0.73$, $P < 0.001$) (Fig. 2b).

Kidney. Renal α-tocopherol levels were approximately 2-fold higher in rats fed vitamin E alone than in

Table I. Changes in Body Weight, Serum Cholesterol and Serum α-Tocopherol Levels in Rats after 10 Weeks on Supplemented Diets

		Dietary group			
		Control	1% cholesterol	0.5% vitamin E	1% Cholesterol + 0.5% vitamin E
n		10	10	10	10
Body weight (kg)	Start	0.40 ± 0.02	0.41 ± 0.02	0.42 ± 0.11	0.44 ± 0.02
	Sacrifice	0.53 ± 0.01	0.52 ± 0.03	0.54 ± 0.12	0.55 ± 0.02
Serum cholesterol (m*M*)	Start	1.58 ± 0.04	1.64 ± 0.11	1.73 ± 0.12	1.63 ± 0.06
	Sacrifice	2.21 ± 0.24	4.04 ± 0.44[a,b]	2.03 ± 0.54	4.28 ± 0.60[a,b]
Serum α-tocopherol (μ*M*)	Start	15.17 ± 2.06	17.3 ± 2.18	17.72 ± 2.55	17.33 ± 2.10
	Sacrifice	21.8 ± 5.90	37.3 ± 5.60	109.18 ± 15.39[c]	123.7 ± 13.0[d]
Serum α-tocopherol/ cholesterol (μm/m*M*)	Start	9.52 ± 1.23	11.76 ± 1.74	12.78 ± 2.18	10.15 ± 1.43
	Sacrifice	10.56 ± 4.72	9.75 ± 3.13	51.38 ± 10.28[c,e]	39.84 ± 7.38[d,e]

Note. Data are means ± SEM.
[a] $P < 0.05$ compared with control by unpaired *t* test.
[b] $P < 0.001$ and [e] $P < 0.05$ by paired *t* test with respect to basal values.
[c] $P < 0.0001$ and [d] $P < 0.001$ compared with cholesterol and control fed rats by unpaired *t* test.

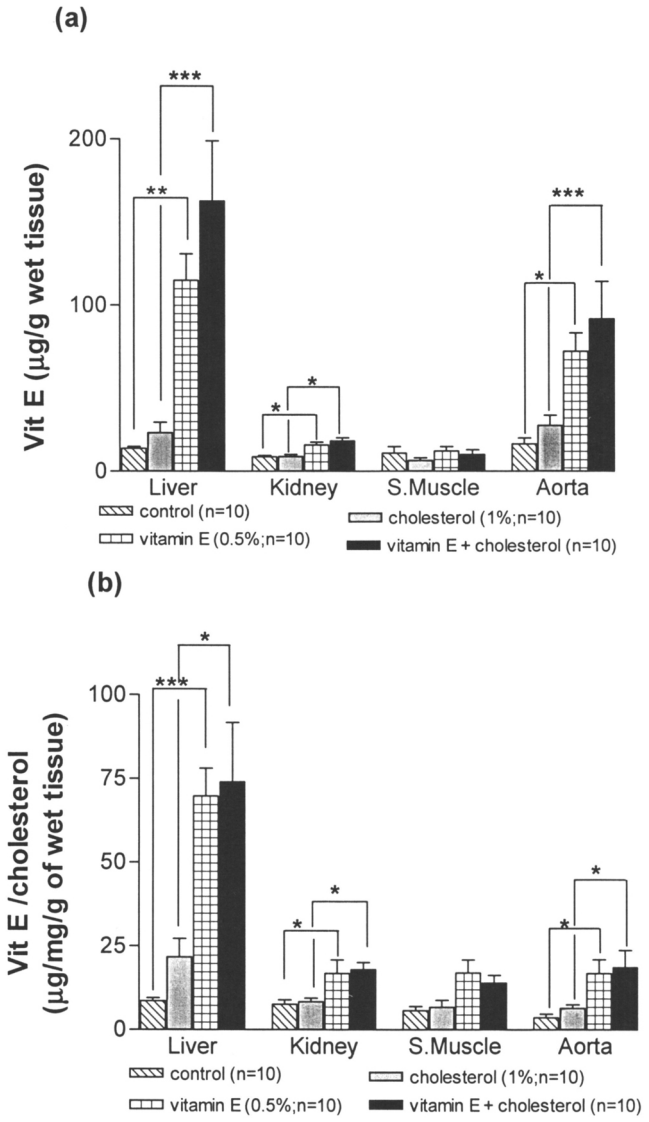

Figure 1. (a) Distribution of α-tocopherol in selected tissues from rats following 10 weeks of dietary supplementation. Bars are means with their standard errors; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. (b) Tissue α-tocopherol/cholesterol ratios in rats after 10 weeks of dietary supplementation. Bars are means with their standard errors; $^{*}P < 0.05$; $^{***}P < 0.001$.

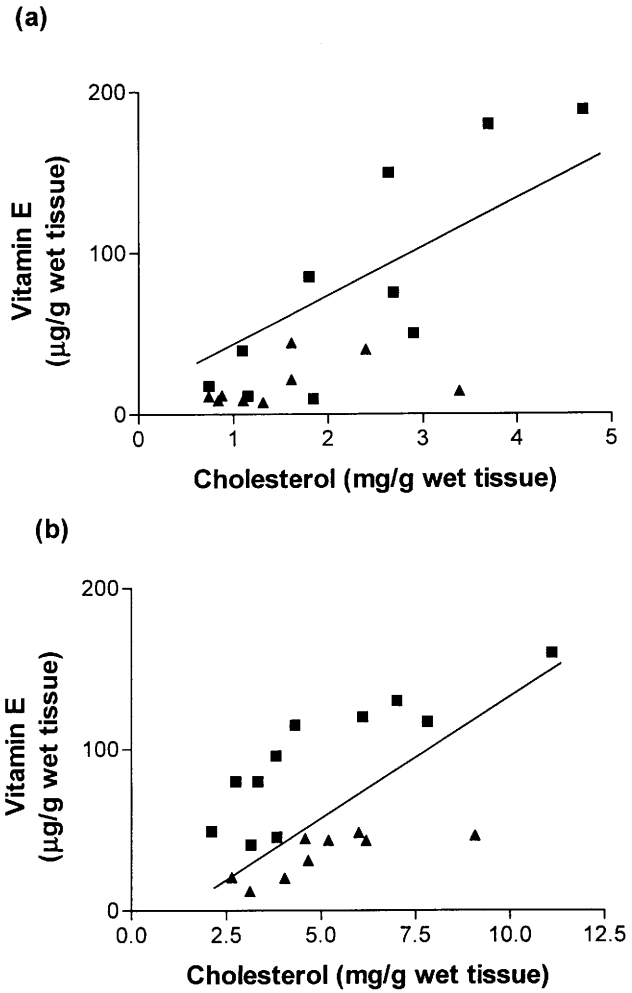

Figure 2. (a) Correlation between hepatic cholesterol and hepatic α-tocopherol in rats after 10 weeks on 1% cholesterol diet (▲; $n = 10$) and 1% cholesterol + 0.5% vitamin E diet (■; $n = 10$); $r = 0.43$; $P = 0.026$. (b) Correlation between aortic cholesterol and aortic α-tocopherol in rats after 10 weeks on 1% cholesterol diet (▲; $n = 10$) and 1% cholesterol + 0.5% vitamin E diet (■; $n = 10$); $r = 0.73$; $P = 0.004$.

those receiving control chow. The same was also the case for rats on the vitamin E plus cholesterol compared with those receiving cholesterol alone ($P < 0.05$ in both cases) (Fig. 1a). A similar pattern was observed when the data were expressed as the α-tocopherol/cholesterol ratio (Fig. 1b).

Skeletal Muscle. Although the skeletal muscle α-tocopherol/cholesterol ratios were higher in animals receiving dietary vitamin E supplementation, these differences failed to reach statistical significance (Fig. 1, a and b).

Discussion

In this study, we investigated the tissue accumulation of α-tocopherol following 10 weeks dietary supplementation. For our studies we chose to feed rats with diets containing 0.5% vitamin E (w/w). In a previous report, Machlin and Gabriel (19) investigated the effects of diets containing 0.1% to 1% vitamin E on plasma and tissue α-tocopherol levels. They reported that plasma concentrations of α-tocopherol reached a plateau by approximately 8 weeks in rats fed a chow diet containing 1% vitamin E. In preliminary experiments we found that plateau levels of α-tocopherol were attained after approximately 10 weeks in animals receiving 0.5% vitamin E with concomitant 1% cholesterol. At this time, we found that levels of serum α-tocopherol had increased 6- to 7-fold above baseline in both groups of animals receiving vitamin E supplemented diets. Mean serum α-tocopherol levels were approximately 14% higher in the animals fed cholesterol plus vitamin E compared with those on vitamin E

alone, but this failed to reach statistical significance. Our data suggest that despite the high dietary content of vitamin E used in the study, the subsequent assimilation of vitamin E was insufficient to saturate the carrier lipoprotein particles in the cholesterol fed animals. Indeed, the mean serum α-tocopherol/cholesterol ratio was higher in the group of animals receiving vitamin E alone than for those on cholesterol plus vitamin E (Table I). Horwitt *et al.* (22) have argued that, because plasma α-tocopherol and lipid levels are strongly correlated, the ratio of plasma α-tocopherol/cholesterol may be a more appropriate index of vitamin E status. We observed a strong positive association between serum α-tocopherol and cholesterol concentrations, and although absolute serum levels of α-tocopherol were raised 2-fold in the animals fed the cholesterol-only diet, the α-tocopherol/cholesterol ratio tended to fall, suggesting that the increase in serum α-tocopherol seen in this group was related to enhanced lipoprotein transport. We did not observe any hypocholesterolaemic effect associated with vitamin E supplementation in the rats, although this has been reported in the rabbit (23). In our study, rats were not treated with cholic acid which facilitates the development of a severe hypercholesterolaemia. In the absence of severe hyperlipidaemia, the lipid lowering effect of vitamin E may be too small to detect.

We observed that dietary vitamin E supplementation, in the presence or absence of concomitant cholesterol feeding, led to an increased hepatic accumulation of α-tocopherol, which exceeded that for the other tissues we investigated. Machlin and Gabriel (19) also noted that the increase in α-tocopherol content of the liver was higher than for other tissues, including aorta, kidney, and skeletal muscle, although Bendich *et al.* (24) found that basal tissue levels of α-tocopherol were lower for the liver than for spleen, testes, adrenal, heart, and cerebrum. The liver is nevertheless the major storage site of α-tocopherol. Hepatic uptake of α-tocopherol has been shown to occur, at least in part, via the LDL receptor pathway (25). Following its uptake by the liver, α-tocopherol is repackaged into other lipoprotein particles including high-density lipoprotein and very low density lipoprotein, and subsequently transported to other tissues (15). Among these tissues is the kidney, a highly vascular and oxygen-dependent organ, which is particularly prone to free-radical injury associated with ischemia-reperfusion and renal transplantation (26, 27). Vitamin E is reported to be protective in these circumstances (26, 27). We observed a 2-fold increase in renal accumulation of α-tocopherol following 10 weeks of dietary vitamin E supplementation (Fig. 1, a and b), and this was also the case when vitamin E was accompanied by a high cholesterol intake. Skeletal muscle is reported to be inherently susceptible to oxidative damage due to its high unsaturated fatty acid content (28).

The antioxidant status of skeletal muscle is dependent on dietary vitamin E intake (28) and vitamin E deficiency is associated with myopathies. We did not observe any significant increase in skeletal muscle α-tocopherol content following 10 weeks dietary supplementation; however, it has been noted previously that vitamin E accumulates in skeletal muscle slowly (19), hence a longer treatment period may be required for any significant differences to become evident.

In animal models of atherosclerosis, the aorta is the primary site of lesion development (29). The basal α-tocopherol content of aortic tissue was higher than that of kidney or skeletal muscle (Fig. 1a). The 1% cholesterol diet was associated with slightly higher absolute α-tocopherol content (Fig. 1a), but an unchanged α-tocopherol/cholesterol ratio (Fig. 1b), suggesting that the increase in α-tocopherol content was attributable to an increase in lipoprotein influx. The absolute aortic α-tocopherol content was highest among the animals receiving dietary vitamin E supplements whether or not this was accompanied by cholesterol supplements. The aortic tissue α-tocopherol/cholesterol ratios were also highest among these groups of animals, indicating that the α-tocopherol accumulation was not simply a reflection of increased lipoprotein uptake. Vitamin E supplements have been shown to inhibit atherosclerotic lesion development in experimental animal models (30, 31), and appear to protect against coronary heart disease in man when taken in sufficient quantities (32, 33). Our data indicate that dietary supplements can increase the alpha-tocopherol content of vascular tissue, causing a potential improvement in the antioxidant reserve of the arterial wall, and thereby increasing its resistance to atherosclerotic lesion formation.

This project was supported by grants from the British Heart Foundation and Ono Pharmaceutical Co., Osaka, Japan.

1. Fritma GA. Vitamin E and auto-oxidation. Am J Med Tech **49**:453–456, 1983.
2. Weiser H, Vecchi M, Schlachter P. Stereoisomers of alpha-tocopheryl acetate. IV. USP units and alpha-tocopherol equivalents of all-rac-, 2-ambo- and RRR-alpha-tocopherol evaluated by simultaneous determination of resorption-gestation, myopathy and liver storage capacity. Int J Vit Nutr Res **56**:45–56, 1986.
3. Behrens WA, Thompson JN, Madere R. Distribution of alpha-tocopherol in human plasma lipoproteins. Am J Nutr **35**:691–696, 1982.
4. Traber MG, Ingold KU, Burton GW, Kayden HJ. Absorption and transport of deuterium-substituted 2R, 4R, 8R-alpha-tocopherol in human lipoproteins. Lipids **23**:791–797, 1986.
5. McCay PB. Vitamin E interaction with free radicals and ascorbate. Annu Rev Nutr **5**:323–340, 1985.

6. Ferns GAA, Konneh M, Anggard EE. Vitamin E: The evidence for an anti-atherogenic role. Artery **20**:61–94, 1993.
7. Janero DR. Therapeutic potential of vitamin E in the pathogenesis of spontaneous atherosclerosis. Free Rad Biol Med **1**:129–144, 1991.
8. Cornwell DG, Huttner JJ, Milo GE, Panganamala PV. Polyunsaturated fatty acids, vitamin E and proliferation of aortic smooth muscle cells. Lipids **14**:194–202, 1979.
9. Forster LA, Li S-R, Nourooz-Zadeh J, Ferns GAA, Anggard EE. Vitamin E supplementation reduces adhesion of mononuclear cells from healthy subjects in vitro. Clin Sci **86**:101P, 1994.
10. Machlin LJ, Bendich A. Free radical tissue damage: Protective role of antioxidant nutrients. FASEB J **1**:441–445, 1989.
11. Esterbauer H, Dieber-Rothender M, Striegl G, Waeg G. Role of vitamin E in preventing the oxidation of low density lipoprotein. Am J Clin Nutr **53**:314S–321S, 1991.
12. Abbey M, Nestel PJ, Baghurst PA. Antioxidant vitamins and low density lipoprotein oxidation. Am J Clin Nutr **58**:525–532, 1993.
13. Konneh M, Nourooz-Zadeh J, Li S-R, Anggard EE, Ferns GAA. Vitamin E inhibits neointimal thickening after balloon angioplasty in cholesterol fed rats. Atherosclerosis **113**:29–39, 1995.
14. Ingold KU, Burton GW, Foster DO, Hughes L, Lindsay DA, Webb A. Biokinetics of, and discrimination between dietary RRR- and SRR-alpha-tocopherols in male rats. Lipids **22**:163–172, 1987.
15. Drevon CA. Absorption, transport and metabolism of vitamin E. Free Rad Res Commun **14**:229–246, 1991.
16. Kaseki H, Kim EY, Whisler RL, Cornwell GD. Effect of an oral dose of vitamin E and cholesterol content of tissues of the vitamin E deficient rat. J Nutr **116**:1631–1639, 1986.
17. Hidiroglou M. Vitamin E levels in sheep tissues at various times after a single oral administration of d-alpha-tocopherol acetate. Int J Vit Nutr Res **57**:381–384, 1987.
18. Jensen M, Lindholm A, Hakkarainen J. The vitamin E distribution in serum, liver, adipose and muscle tissues in the pig during depletion and repletion. Acta Vet Scand **31**:129–136, 1990.
19. Machlin LJ, Gabriel E. Kinetics of tissue alpha-tocopherol uptake and depletion following administration of high level of vitamin E. Ann New York Acad Sci **393**:48–60, 1982.
20. Stewart-Lee AL, Forster LA, Nourooz-Zadeh J, Ferns GAA, Anggard EE. Vitamin E protects against impairment of endothelium-mediated relaxations in cholesterol-fed rabbits. Arterioscl Thromb **14**:494–499, 1991.
21. Trinder P. Oxidase determination of plasma cholesterol as cholest-4-ene-3-one using iso-octane extraction. Ann Clin Biochem **18**:64–70, 1981.
22. Horwitt MK, Harvey CC, Dahm CH Jr., Searcy MT. Relationship between tocopherols and serum lipid levels for determination of nutritional adequacy. Ann NY Acad Sci **203**:223–236, 1972.
23. Sundaram GS, London R, Manimekalai S, Nair PP, Goldstein P. Alpha-Tocopherol and serum lipoproteins. Lipids **16**:223–227, 1981.
24. Bendich A, Gabriel E, Machlin LJ. Differences in vitamin E levels in tissues of the spontaneously hypertensive and Wistar-Kyoto rats (41560). Proc Soc Exp Biol Med **172**:297–300, 1983.
25. Cohn W, Goss-Sampson MA, Grun H, Muller DP. Plasma clearance and net uptake of alpha-tocopherol and low density lipoprotein by tissues in WHHL and control rabbits. Biochem J **287**:247–254, 1992.
26. Princemail J, DeFraigne JO, Franssen C, Bonnet P, Deby-Dupont G, Pirenne J, Deby C, Lamy M, Limet M, Meurisse M. Evidence for free radical formation during human kidney transplantation. Free Rad Biol Med **15**:343–348, 1993.
27. Demirbas A, Bozoklu S, Ozdemir A, Bilgin N, Haberal M. Effect of tocopherol on perfusion injury caused by free radicals in canine kidney autotransplantation model. Transplant Proc **25**:2274, 1993.
28. Chan KM, Decker EA. Endogenous skeletal muscle antioxidants. Crit Rev Food Sci Nutr **34**:403–426, 1994.
29. Faggiotto A, Ross R, Harker L. Studies of hypercholesterolemia in non-human primate. I. Changes that lead to fatty-streak formation. Arteriosclerosis **4**:323–340, 1984.
30. Verlangieri AJ, Bush MJ. Effects of d-alpha-tocopherol supplementation on experimentally-induced primate atherosclerosis. J Am Coll Nutr **11**:131–138, 1992.
31. Williams RJ, Motteram JM, Sharp CH, Gallaher PJ. Dietary vitamin E and the attenuation of early lesion development in modified Watanabe rabbits. Atherosclerosis **94**:153–159, 1992.
32. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willet WC. Vitamin E consumption and risk of coronary heart disease in men. N Engl J Med **328**:1450–1456, 1993.
33. Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willet WC. Vitamin E consumption and risk of coronary disease in women. N Engl J Med **328**:1444–1449, 1993.