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Effects of chronic vitamin E deficiency and a high polyunsaturated fatty acid diet on rat mesenteric arterial function

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1 Male rats were deprived as weanlings of dietary vitamin E and fed on a high polyunsaturated fatty acid (PUFA) diet for 6 months. Rats fed on a high PUFA or on an untreated diet served as controls. Mesenteric arterial beds were isolated and perfused at a constant flow rate (5 ml min^{-1}) and the function of sympathetic nerves, smooth muscle and endothelium was assessed.

2 Electrical field stimulation (4-32 Hz, 90 V, 1 ms, for 30 s) elicited frequency-dependent vasoconstriction of the mesenteric arterial preparations. Response curves were similar between untreated control and PUFA-fed control groups. Maximum vasoconstrictor responses (at 24 and 32 Hz) were significantly attenuated in rats deprived of vitamin E and on a high PUFA diet compared to the PUFA-fed controls ($P<0.05$).

3 Exogenous noradrenaline (NA;0.15-500 nmol) elicited dose-dependent constriction of the mesenteric arterial beds. Preparations from rats fed on a high PUFA diet elicited significantly smaller responses compared to the control group. There was no significant difference in constrictor responses of PUFA rats deprived of vitamin E compared to the PUFA controls. Vasoconstrictor responses to doses of adenosine 5'-triphosphate (ATP) (5-5000 nmol) were significantly impaired in vitamin E-deficiency with a high PUFA diet compared to a high PUFA diet alone ($P<<0.001$). Constrictor responses to potassium chloride (0.15 mmol) were significantly impaired in vitamin E-deficient PUFA rats compared to the PUFA-fed control group ($P<0.05$).

4 Vasodilator responses were assessed in preparations in which tone was raised by continuous perfusion with methoxamine (4-25 μM). Mesenteric arterial beds from PUFA-fed rats deprived of vitamin E acquired significantly less tone, 59.8 ± 4.6 mmHg ($n=7$), than PUFA-fed controls 116.9 ± 7.6 mmHg ($n=7$) ($P<0.001$) and were refractory to further increases in tone with further additions of methoxamine. Methoxamine-induced tone of PUFA-fed controls was greater than in P that in the untreated controls (83.9 ± 7.4 mmHg; $n=5$) ($P<0.05$). Responses to the endothelium-dependent vasodilators acetylcholine (ACh) and ATP were significantly reduced in preparations from rats fed on the vitamin E-deficient high-PUFA diet compared to PUFA controls. Vasodilator responses to ACh were greater in PUFA controls than in untreated controls and this reached statistical significance at 5 nmol ACh.

5 Vasodilator responses to sodium nitroprusside, which acts directly on the vascular smooth muscle, were similar in untreated control and PUFA control groups. Responses were significantly attenuated in vitamin E-deficient PUFA rats compared to the PUFA control group ($P<<0.001$).

6 These results indicate that a combination of a high PUFA diet and vitamin E deficiency impairs mesenteric arterial function at the level of the vascular smooth muscle. A high PUFA diet alone attenuates responses to NA and augments endothelium-dependent vasodilatation. The detrimental effects of loss of antioxidant activity due to vitamin E-deficiency on vascular function may be exacerbated by a high PUFA diet.

Keywords: Endothelium; polyunsaturated fatty acids; rat mesenteric arteries; vitamin E deficiency

Introduction

Much damage is known to occur to biological tissues as a result of the action of highly reactive free radical species formed during various biological reactions as well as in ageing and in the development of certain chronic diseases (Eichholzer *et al.*, 1992; Packer, 1992; Simonoff *et al.*, 1992; Rice-Evans & Burdon, 1993). An important target of free radicals is the biomembrane and polyunsaturated fatty acids (PUFAs) appear to be particularly vulnerable due to the susceptibility of their side-chains to attack (Halliwell & Chirico, 1993). Vitamin E, comprising a family of naturally-occurring lipid-soluble essential antioxidants (tocopherols and tocotrienols), has a crucial role in maintaining the integrity and stability of biological membranes; it acts as part of a complex system of biological antioxidants (including ascorbate, dihydrolipoate and electron transporting systems), to quench peroxyl radicals thus interrupting the chain-propagating process of peroxyl radical formation (Burton *et al.*, 1983; Muller & Goss-Sampson, 1990). In addition, vitamin E modulates processes such as the formation of prostanoids, hydroxyeicosatetraenoic acid and sterols (Packer, 1992).

Vitamin E deficiency is associated with ataxia, areflexia, loss of proprioception, ophthalmoplegia, pigmentary retinopathy and generalized muscle weakness (Muller *et al.*, 1983; Harding, 1987; Sokol, 1990). Vitamin E deficiency in humans and experimental animals causes neurological dysfunction involving central and peripheral nervous systems (Goss-Sampson *et al.*, 1990; Sokol, 1990). In rats impaired endothelium-dependent relaxation has been described in the aorta (Rubino & Burnstock, 1994) and mesenteric arteries (Hubel *et al.*, 1989) after deprivation of dietary vitamin E for 4 and 9 months respec-

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tively. We have shown impaired endothelium-dependent relaxation of the rat mesenteric arterial bed at 12 months after dietary vitamin E deprivation, but no change at 6 months (Ralevic *et al.*, 1995).

Diets high in plasma cholesterol-lowering PUFAs, particularly n-3 fatty acids, lower the number of low-density lipoproteins (LDL), have antithrombotic and anti-inflammatory properties, and may play an important role in the prevention and treatment of vascular and inflammatory diseases (Simopoulos, 1991). On the other hand, diets high in PUFAs may increase the susceptibility of LDL to oxidative modification, particularly when mechanisms involved in the protection of cells from lipid peroxidation are overwhelmed (Harats *et al.*, 1991; Oostenbrug *et al.*, 1993). Thus, the therapeutic benefits of dietary PUFAs may be considerably affected by antioxidant status. The atherogenic potential of LDLs strongly increases if their PUFAs undergo peroxidation (Esterbauer *et al.*, 1990; Rice-Evans & Burdon, 1993; Esterbauer, 1993) and arteriosclerosis-like lesions have been observed during chronic marginal deficiency of vitamin E in several animal species (Gey *et al.*, 1991; Steinberg, 1991; Gey, 1992). Vascular responses to catecholamines and angiotensin II have been shown to be reduced, unaffected, or even increased by dietary PUFA (Lockette *et al.*, 1982; Kenny *et al.*, 1990; Yin *et al.*, 1991; Chu *et al.*, 1992; Macleod *et al.*, 1994). In rat mesenteric arteries responses to exogenous noradrenaline (NA) and sympathetic nerve stimulation were attenuated following feeding with dietary fish oils (rich in n-3 PUFA) (Yin *et al.*, 1991; Chu *et al.*, 1992). An increase in endothelium-dependent relaxation as a consequence of a PUFA diet has been described (Shimokawa & Vanhoutte, 1988).

The aim of the present study was to examine the effects of a diet high in PUFA given in the absence of adequate antioxidant protection produced by a deficiency of vitamin E. To this end mesenteric arterial beds were isolated from 6-month-old rats fed as weanlings on a 10% corn oil (n-6 PUFA) diet with normal vitamin E or in the absence of vitamin E. The function of sympathetic nerves, smooth muscle (vasoconstrictor and vasodilator responses) and endothelium was assessed.

Methods

Animals and diet

Weanling (21–23 day), pathogen-free, male Wistar rats were obtained from Charles Rivers Limited, U.K. Two groups of control rats were fed on either a normal diet or a high PUFA diet (both containing vitamin E). The high-PUFA diet was a 10% corn oil diet of the following composition (g kg^{-1}): vitamin free diet casein 200, dextrose 653.2, tocopherol stripped corn oil (stabilized with 0.02% BHT) 100, choline bitartrate 1.8, vitamin mix 302362 5.0, salt mix 200650 40.0 and α-tocopherol acetate 0.04. Vitamin E deficient rats received the same PUFA diet with the omission of vitamin E. The vitamin E content of this diet was determined to be less than 1 ng ml^{-1}.

All diets were stored in the dark at 4°C until use. Diets were made specifically to order and were supplied by Dyets Inc. (Pennsylvania, U.S.A.). Food and water were provided *ad libitum*. Rats were used at 6 months. Data for the 6 month control rats (untreated diet) also appears in a sister paper on the effects of vitamin E-deficiency with age (Ralevic *et al.*, 1995).

Isolated mesenteric arterial bed preparation

Rats were killed by an overdose of pentobarbitone (sagatal, 60 mg kg^{-1} i.p.). Mesenteric beds were isolated from the rats and set up for perfusion as described previously (Ralevic *et al.*, 1993). The abdomen was opened and the superior mesenteric artery exposed and cannulated with a hypodermic needle. The superior mesenteric vein was severed and blood was gently flushed through the bed with 1 ml Hanks solution. The gut was dissected away and the preparation mounted on a stainless steel grid (7 × 5 cm) in a humid chamber (custom-made at University College London). The preparation was perfused at a constant flow rate of 5 ml min^{-1} using a peristaltic pump (model 7554-30, Cole-Parmer Instrument Co., Chicago Illinois). The perfusate was Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_2 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, gassed with 95% O_2–5% CO_2 and maintained at 37°C. Responses were measured as changes in perfusion pressure (mmHg) with a pressure transducer (model P23XL, Viggo-Spectramed, Oxnard, CA) on a side-arm of the perfusion cannula, and recorded on a polygraph (model 7D, Grass Instrument Co., Quincy, Mass). Electrical field stimulation of perivascular nerves was achieved by passing a current (stimulator model SD9, Grass) across the preparation between the cannulation needle and the wire grid on which the preparation rested. Preparations were allowed to equilibrate for 30 min before experimentation.

Stimulation of perivascular nerves

Electrical field stimulation of perivascular nerves was achieved by passing a current across the preparation between the cannulation needle and the wire grid on which the preparation rested. Stimulation at basal tone (unconstricted preparation) (90 V, 1 ms, 4–32 Hz, for 30 s) elicited vasoconstrictor responses which could be abolished by guanethidine (5 μM), confirming that these resulted from stimulation of perivascular sympathetic nerves.

Experimental protocol

Electrical field stimulation at a range of frequencies (4–32 Hz) was carried out to allow a frequency-response curve for sympathetic nerves to be constructed. Vasoconstrictor responses of preparations to doses of the sympathetic transmitters NA and ATP were then assessed. The tone of the preparations was raised by continuous perfusion with methoxamine (4–25 μM) to allow vasodilator responses to doses of acetylcholine (ACh), ATP and sodium nitroprusside (SNP), in that order, to be established. Responses of preparations to a dose of KCl (0.15 mmol) were determined at the end of each experiment after all drugs had been washed out and the preparations allowed to equilibrate for 15 min.

Drugs

All drugs were applied as 50 μl bolus injections into a rubber septum proximal to the preparation unless otherwise stated. Drugs were made up daily in distilled water except for NA which was made up as a stock solution of 10 mM in 0.1 mM ascorbic acid. Noradrenaline (arterenol bitartrate), methoxamine hydrochloride, adenosine 5′-triphosphate (ATP, disodium salt), acetylcholine chloride and sodium nitroprusside were all obtained from Sigma, Poole, U.K.

Data analysis

Responses were measured as changes in perfusion pressure (mmHg). Vasodilator responses were evaluated as a percentage of the methoxamine-induced increase in tone. Results are presented as the mean ± s.e.mean. Differences between the groups were measured by analysis of variance with repeated measures, with the level of significance set at $P<0.05$. Post hoc *t* test with Bonferroni correction was used to see where the differences lay. With Bonferroni correction in this planned comparison (untreated control *versus* PUFA-control and vitamin E-deficient control *versus* PUFA-control) results were considered significant when $P<0.025$.

Results

Animals

Experimental rats which had been on the vitamin E-deficient PUFA diet for 6 months following weaning weighed significantly less (406.4±21 g, $n=8$) than the age-matched PUFA- controls (637.4±27 g, $n=8$) ($P<0.001$). The PUFA-treated group was significantly heavier than the untreated controls (512±10 g, $n=6$) ($P<0.001$). The vitamin E-deficient rats exhibited poor coat condition, muscle wasting, kyphoscoliosis, hind limb weakness and impaired gait as previously described (Goss-Sampson *et al.*, 1990).

Base-line parameters

There was no significant difference in basal mesenteric arterial perfusion pressure between the groups: vitamin E-deficient PUFA rats, 32.6±1.3 mmHg ($n=8$); PUFA-controls, 30.5±1.8 mmHg ($n=8$); untreated controls, 31.9±1.6 ($n=6$). Methoxamine (4–25 μM) produced a significantly smaller increase in tone in mesenteric beds from vitamin E-deficient PUFA-fed rats (59.8±4.6 mmHg, $n=7$) than in PUFA-control preparations (116.9±7.6 mmHg, $n=7$, $P<0.001$). Further addition of methoxamine did not produce a further increase in tone in mesenteric beds from vitamin E-deficient PUFA-fed rats. Methoxamine induced a smaller increase in tone in the untreated controls (83.9±7.4 mmHg, $n=5$) than in the PUFA-control group ($P<0.05$).

Vasoconstrictor responses to stimulation of sympathetic nerves

Electrical field stimulation of perivascular sympathetic nerves elicited frequency-dependent vasoconstrictor responses. There was a significant difference between the groups ($P<<0.001$). Responses of the vitamin E-deficient PUFA group were significantly smaller than those of the PUFA-control group at the highest frequencies of stimulation (24 and 32 Hz) ($P<0.05$) (Figure 1). Responses between the control and PUFA-control groups were similar (Figure 2).

Vasoconstrictor responses to exogenous NA, ATP and KCl

Bolus injections of NA elicited dose-dependent vasoconstrictor responses in the mesenteric arterial bed preparations. There was a significant difference between the groups ($P=0.001$). Responses of the PUFA-fed control group were significantly less than those of the untreated controls (Figure 3). Responses of preparations from the vitamin E-deficient PUFA-fed rats were similar to those of the PUFA-fed controls (Figure 3).

ATP (5–5000 nmol) elicited dose-dependent vasoconstriction, which was significantly impaired in vitamin E-deficient PUFA-fed rats compared to responses of the PUFA-controls ($P<<0.001$) (Figures 1 and 4).

Vasoconstrictor responses of mesenteric beds to KCl (0.15 mmol) were significantly attenuated in mesenteric beds from vitamin E-deficient rats compared to the PUFA-control group. Responses were 24.0±4.47 mmHg ($n=7$) in preparations from vitamin E-deficient PUFA-fed rats and 74.29±20.12 mmHg ($n=8$) in PUFA-fed controls ($P<0.05$). Responses of the untreated controls, 57.5±7.54 mmHg ($n=6$), and the PUFA-fed controls were similar.

Endothelium-dependent vasodilator responses to ACh and ATP

There was a significant difference in relaxant responses to ACh between the groups ($P<0.01$). Dose-dependent relaxant responses to ACh were significantly smaller in absolute terms than those of the PUFA-fed control groups, although the de-

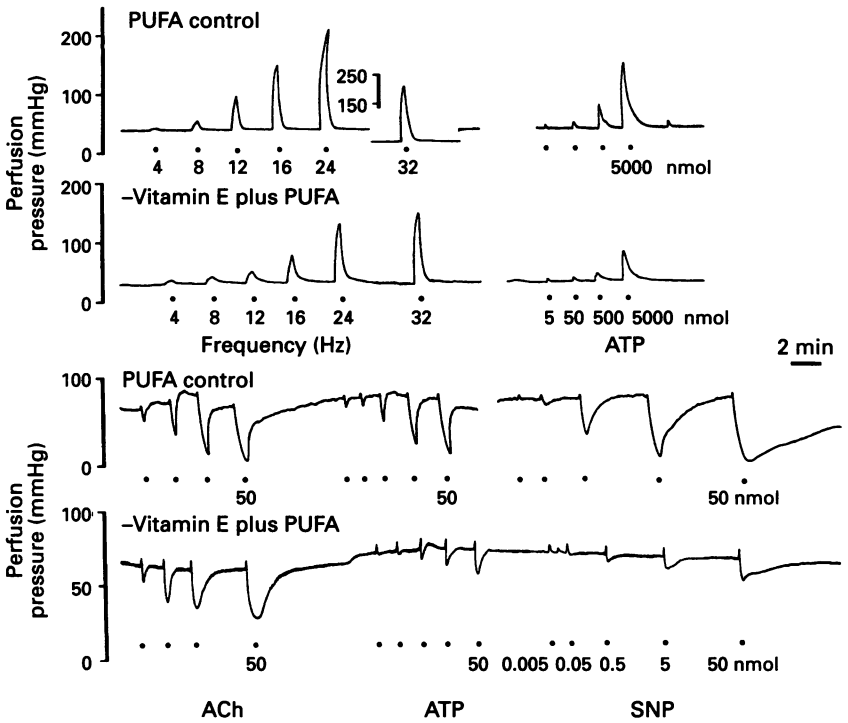

Figure 1 Representative traces showing responses of mesenteric arterial preparations from polyunsaturated fatty acid (PUFA)-fed control and vitamin E-deficient PUFA-fed rats at basal and raised tone. (a) Basal tone: frequency-dependent vasoconstriction to electrical field stimulation (Frequency, Hz) and dose-dependent vasoconstriction to ATP (5–5000 nmol) was significantly attenuated in preparations from vitamin E-deficient PUFA-fed rats. Note that at 32 Hz in the PUFA-fed control group the scale has been changed. (b) Raised tone: Endothelium-dependent vasodilatation to acetylcholine (ACh) and ATP and endothelium-independent vasodilatation to sodium nitroprusside (SNP) (all at 0.005–50 nmol) was impaired in vitamin E-deficiency plus PUFA compared to the PUFA-fed controls.

gree of methoxamine-induced increase in tone was also significantly smaller. When expressed as a percentage of methoxamine-induced tone this statistical difference was retained (Figures 1 and 5a).

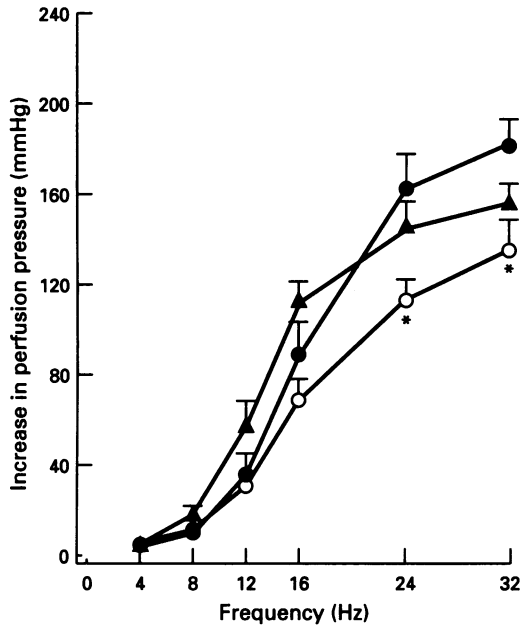

Figure 2 Frequency-response curves showing vasoconstrictor responses (increase in perfusion pressure, mmHg) of rat mesenteric arterial beds to electrical field stimulation of sympathetic nerves (4–32 Hz, 90 V, 1 ms, for 30 s). (●) Polyunsaturated fatty acid-fed (PUFA)-control rats ($n=8$). (○) PUFA-vitamin E-deficient rats ($n=8$). (▲) Untreated control rats ($n=6$). A statistically significant difference between the PUFA-control group and PUFA-vitamin E-deficient group is denoted by $*P<0.025$.

Dose-dependent vasodilatation to ATP was significantly reduced in mesenteric arterial beds from vitamin E-deficient PUFA-fed rats compared to the PUFA-fed controls ($P<<0.001$) (Figures 1 and 5b).

Endothelium-independent vasodilator responses to SNP

There was a significant difference between the groups for endothelium-independent vasodilatation to SNP ($P<<0.001$). Responses of the vitamin E-deficient PUFA-fed group were significantly smaller than those of the PUFA-control group (Figures 1 and 6). Responses between the untreated control group and the PUFA-fed control group were similar (Figure 6).

Discussion

This study was designed to examine the consequence of a high PUFA diet on vascular function during inadequate antioxidant protection due to deprivation of dietary vitamin E. We have previously shown that deprivation of dietary vitamin E for 6 months after weaning has no significant effect on mesenteric arterial function (sympathetic nerves, endothelium and vascular smooth muscle) compared to rats maintained on a normal diet (Ralevic *et al.*, 1995). Hence, any changes seen in the present study during vitamin E-deficiency and PUFA supplementation are attributable to the effects of the high PUFA-diet in combination with vitamin E-deficiency.

Vitamin E is known to be important for the maintenance of normal neural structure and function (Goss-Sampson *et al.*, 1990; Muller & Goss-Sampson, 1990; Sokol, 1990; Schmidt *et al.*, 1991). The particular susceptibility of neural tissues to impairment in vitamin E deficiency has been discussed in terms of the vulnerability of the extended surface area of the neuronal membrane and remoteness of the nerve ending from the cell body (Muller & Goss-Sampson, 1990). The reduced vasoconstrictor responses to electrical field stimulation of sympathetic nerves in the vitamin E-deficient high-PUFA group

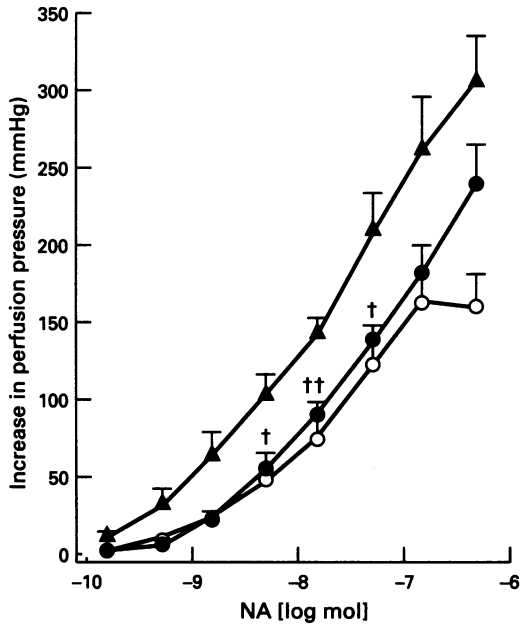

Figure 3 Dose-response curves showing vasoconstrictor responses (increase in perfusion pressure, mmHg) of rat mesenteric arterial beds to exogenous noradrenaline (NA). (●) Polyunsaturated fatty acid-fed (PUFA)-control rats ($n=8$). (○) PUFA-vitamin E-deficient rats ($n=8$). (▲) Untreated control group ($n=6$). Statistically significant differences between the untreated control group and the PUFA-fed control group are denoted by $\dagger P<0.025$, $\dagger\dagger P<0.005$.

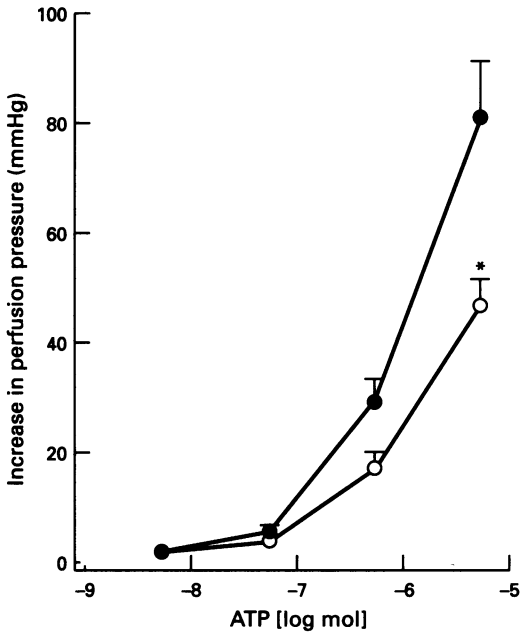

Figure 4 Dose-response curves showing vasoconstrictor responses (increase in perfusion pressure, mmHg) of rat mesenteric arterial beds to exogenous ATP. (●) Polyunsaturated fatty acid-fed (PUFA)-control rats ($n=8$). (○) PUFA-vitamin E-deficient rats ($n=8$). Statistically significant difference between the groups are denoted by $*P<0.025$.

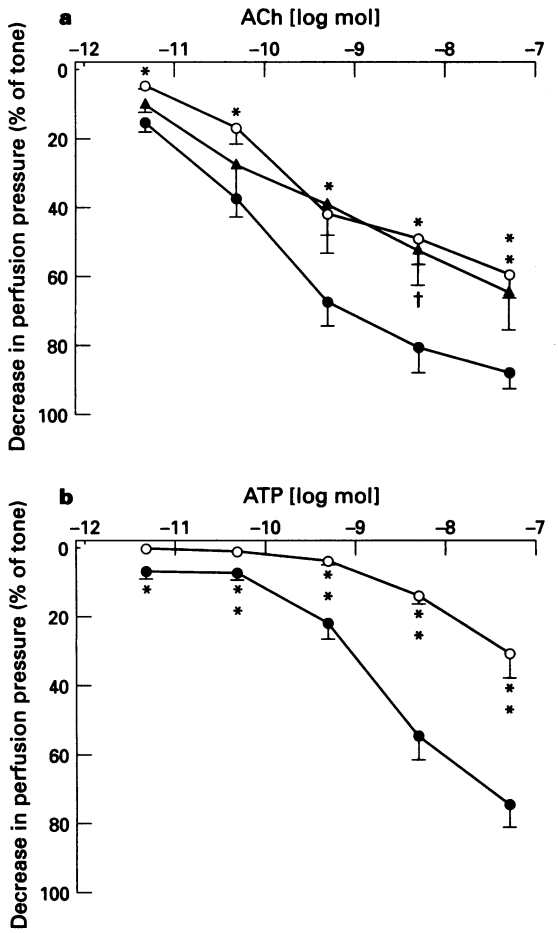

Figure 5 Dose-response curves showing vasodilator responses (decrease in perfusion pressure, % of tone) of rat mesenteric arterial beds to (a) acetylcholine (ACh), (b) adenosine 5'-triphosphate (ATP). (●) Polyunsaturated fatty acid-fed (PUFA)-control rats ($n=7$). (○) PUFA-vitamin E-deficient rats ($n=7$). (▲) Untreated control rats ($n=5$). A statistically significant difference between PUFA-vitamin E-deficient and PUFA-control groups is denoted by $*P<0.025$ and $**P<0.005$, and between PUFA-control and untreated control groups by $\dagger P<0.025$.

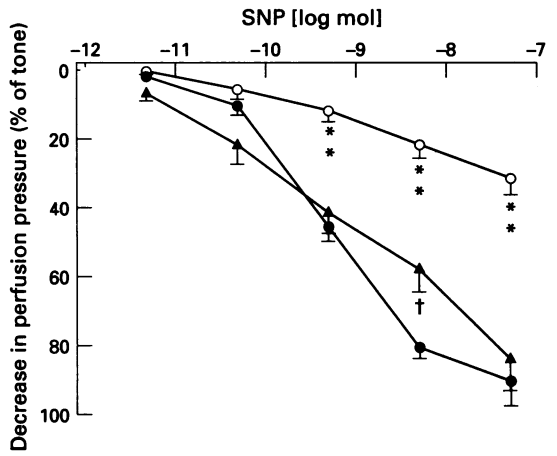

Figure 6 Dose-response curves showing vasodilator responses (decrease in perfusion pressure, % of tone) of rat mesenteric arterial beds to exogenous sodium nitroprusside (SNP). (●) Polyunsaturated fatty acid-free (PUFA)-control rats ($n=7$). (○) PUFA-vitamin E-deficient rats ($n=7$). (▲) Untreated control rats ($n=5$). A statistically significant difference between: PUFA-vitamin E-deficient and PUFA-control groups is denoted by $**P<0.01$ and between PUFA-control and untreated control groups is denoted by $\dagger P<0.025$.

seen in the present study may indicate sympathetic malfunction. Since no such change was observed in a vitamin E-deficient diet alone this is attributable to the detrimental effects of a high PUFA diet. This is consistent with previous findings showing that diets rich in PUFAs worsen nervous system injury due to vitamin E deficiency (Dam, 1944). However, we cannot exclude the possibility that impaired sympathetic constriction was due to a decrease in the constrictor potential of the vascular smooth muscle, since responses to methoxamine, ATP and KCl were also impaired. Further studies measuring the release of catecholamines are required to see if prejunctional mechanisms are involved.

In man dietary PUFA may reduce blood pressure (Bonaa, 1989) and in other animals vascular responses to catecholamines and angiotensin II have been shown to be reduced, unaffected, or even increased (Lockette *et al.*, 1982; Kenny *et al.*, 1990; Yin *et al.*, 1991; Chu *et al.*, 1992; MacLeod *et al.*, 1994). In the present study a decrease in responsiveness to NA in the PUFA-fed group compared to the untreated controls was observed. This is compatible with previous reports of attenuated responses of the isolated rat mesenteric arterial bed to exogenous NA and to sympathetic nerve stimulation following feeding with dietary fish oils (rich in n-3 PUFA); there was no significant effect on responses to vasopressin or KCl (Yin *et al.*, 1991; Chu *et al.*, 1992). A degree of selectivity in this effect is also indicated in our study since there was no significant difference in constriction to KCl between the PUFA control and untreated control groups. Unfortunately, control data for ATP are not available so we are unable to say whether attenuation of responses occurs for substances other than NA. More recently, dietary n-3 but not n-6 PUFA supplements were shown to attenuate contractile responses of rat femoral resistance arteries to NA (MacLeod *et al.*, 1994).

The mechanism by which vitamin E-deficiency during a high PUFA diet causes attenuation of smooth muscle constriction was not determined, but clearly this is not a receptor-specific effect. It is possible that impaired constriction was due to an increase in lipid peroxidation, altering membrane fluidity and permeability, inactivating membrane-bound receptors and increasing non-specific permeability to ions (Halliwell & Chirico, 1993). The phytyl side-chain of vitamin E may be important in membrane stabilization (Burton *et al.*, 1983). In addition, breakdown products of lipid peroxidation can further damage cellular function, particularly via effects on proteins. Decreased intestinal smooth muscle responsiveness to muscarinic and β-adrenoceptor agonists by lipid peroxidation products *in vitro* has been described (Van der Vliet *et al.*, 1991).

The high PUFA diet alone appeared to enhance endothelium-dependent relaxation compared to the untreated control group. Such an enhancement could provide an explanation for the decreased constrictor responses to NA during a high PUFA diet as shown by us and others, since the endothelium has an inhibitory modulatory effect on NA-mediated constrictor function. The release of endothelial relaxing factors has previously been suggested to be increased in animals fed on PUFA diets (Shimokawa & Vanhoutte, 1988) and increases in prostacyclin (PGI_2) have been linked to intake of n-3 fatty acids in human vessels (DeCaterina *et al.*, 1990). Our conclusions regarding enhanced endothelial function are based on results obtained with ACh alone, and it is unfortunate that control data for ATP is not available to test this further.

Endothelium-dependent vasodilatation to ACh and ATP in vitamin E-deficiency with PUFA was significantly impaired compared to the PUFA-control diet alone. Previous studies have described endothelial malfunction due to long-term vitamin E-deficiency on an otherwise normal diet in rat aorta and mesenteric arteries (Hubel *et al.*, 1989; Rubino & Burnstock, 1994). Since vasodilator responses to both ACh and ATP were decreased this suggests that endothelial malfunction is not due to a defect in a specific endothelial receptor. Impaired vasodilatation to ACh and ATP may be a consequence of peroxidation of lipids and altered function of the endothelial cell membrane (Halliwell & Chirico, 1993; Van Acker *et al.*,

1993). Changes in endothelial signal transduction, which for ACh and ATP in rat mesenteric arteries involves nitric oxide and endothelium-derived hyperpolarizing factor (Ralevic & Burnstock, 1991; Parsons *et al.*, 1994), may also be involved. On the other hand, increased levels of a cyclo-oxygenase-dependent vasoconstrictor has been shown in mesenteric arteries of rats after 10 weeks of vitamin E deficiency (Davidge *et al.*, 1993), and could contribute to attenuation of endothelium-dependent relaxation. Impaired endothelium-dependent relaxation may also be a consequence of the effects of oxidized LDLs which impair relaxations to endothelium-dependent and -independent agonists *in vitro*.

Since vasodilator responses to SNP were also significantly attenuated in vitamin E deficiency it is possible that impaired smooth muscle relaxation is the underlying cause of attenuated relaxations to ACh and ATP. A change in the vascular smooth muscle due to lipid peroxidation (Halliwell & Chirico, 1993; van Acker *et al.*, 1993) may be the cause of attenuated vasodilatation as suggested above for impaired vasoconstriction. It is notable that relaxant responses to SNP were more attenuated than those to ACh. It is possible that the experimental protocol, which involved testing ACh, ATP and SNP in that order, was critical and that our results represent a deterioration of the preparation with time.

We have previously shown that, in rats, 6 months of vitamin E-deficiency alone does not elicit any functionally-expressed changes in sympathetic nerves, smooth muscle or endothelium of the mesenteric arteries (Ralevic *et al.*, 1995). Clearly, in the present study it is the combination of the high PUFA diet and vitamin E deficiency which causes the impairment of smooth muscle vasoconstrictor and vasodilator function. To what extent the impaired sympathetic vasoconstriction and endothelium-dependent vasodilatation are functions of these changes in the smooth muscle is not clear. The mechanism by which this combination produced such severe changes was not studied. However, it is possible that this is due to a destructive combination of changes in the cell membrane phospholipid fatty acid composition caused by the high PUFA diet and lipid peroxidation due to vitamin E-deficiency.

In conclusion, the results of the present study show that mesenteric arterial function is impaired in rats fed on a high PUFA diet and deprived of dietary vitamin E. These detrimental effects of vitamin E deficiency negate the potentially beneficial effects of a high PUFA diet on vascular function. These results are consistent with the hypothesis that vitamin E is crucial for the maintenance of normal biological function. In addition, the balance of dietary factors and biological antioxidant status is important. With considerable interest in the use of high PUFA diets in the prevention and treatment of various pathological conditions the potentially harmful effects should be considered.

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