Diabetologia

Early vitamin E supplementation attenuates diabetes-associated vascular dysfunction and the rise in protein kinase C-β in mesenteric artery and ameliorates wall stiffness in femoral artery of Wistar rats

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Abstract

Aims/hypothesis. The impact of early vitamin E supplementation on vascular function in diabetes remains unresolved. Therefore, we examined the effects of vitamin E on functional and structural parameters and on chemical markers that are disturbed in diabetes in mesenteric and femoral arteries.
Methods. Segments of both arteries, taken from control and 8-week-old streptozotocin diabetic Wistar rats that were treated or not with vitamin E, were mounted on wire and pressure myographs, after which endothelium-dependent and -independent vasodilation was assessed. Passive mechanical wall properties and the localisation and levels of protein kinase C (PKC)-β_2 and AGE were evaluated in these vessels.
Results. Vitamin E supplementation was associated with improved endothelium-dependent and -independent vasodilatation in mesenteric arteries from diabetic rats. Impaired endothelium-dependent vasodilatation in diabetic mesenteric vessels was associated with PKC-β_2 up-regulation and this was prevented by vitamin E supplementation. Increased AGE accumulation and plasma isoprostane levels in diabetic rats were not changed by vitamin E. In the femoral artery, vitamin E supplementation had no effect on endothelium-dependent or -independent vasodilatation, but did prevent the wall stiffening associated with diabetes.
Conclusions/interpretation. Early vitamin E supplementation has a beneficial effect on diabetes-induced endothelial dysfunction in resistance arteries. This benefit may arise from a direct effect on smooth muscle function, as a result of inhibition of the PKC-β_2 isoform by vitamin E.

Keywords Advanced glycation end-products · Diabetes · Endothelial function · Endothelium-derived hyperpolarising factor · Femoral artery · Mesenteric artery · Protein kinase C · Sodium nitroprusside · Stress–strain relationship · Vitamin E

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Abbreviations: ACh, acetylcholine · EDHF, endothelium-derived hyperpolarising factor · GHb, glycohaemoglobin · h, wall thickness · ID, internal diameter · L, length · l-NAME, N$^\omega$-nitro-L-arginine methyl ester · NO, nitric oxide · OD, outside diameter · P, intraluminal pressure · PKC, protein kinase C · PSS, physiological saline solution · SNP, sodium nitroprusside · STZ, streptozotocin · VitE, vitamin E supplementation · V$_{max}$, maximal relaxation

Introduction

Abnormal vascular reactivity is a hallmark of the complications of diabetes [1, 2]. Increased oxidative stress, as a result of hyperglycaemia, impairs endothelium-dependent relaxation [3] and leads to vascular wall thickening via vascular smooth muscle hypertrophy and hyperplasia [4], and to stiffening through the formation of AGE [5]. Vitamin E decreases protein kinase C (PKC) activation [6], a key mediator of the vasoconstrictor response to oxidative stress [7]. However, the benefits of vitamin E supplementation (VitE) in diabetes are variable, with improved endothelium-dependent vasodilatation seen in conduit arteries in streptozotocin (STZ)-induced diabetic rats [8]. Low dose VitE did not reverse vasodilator dysfunction in

mesenteric arteries from STZ-diabetic rats [9]. In one small study, VitE improved flow-mediated dilatation in the brachial artery of subjects with Type 1 diabetes, while it had no effect on arterial compliance [10].

Although one randomised placebo-controlled clinical trial has demonstrated that VitE reduces the incidence of cardiovascular events in patients with established cardiovascular disease [11], two recent prospective clinical trials found no benefit from VitE [12, 13]. However, there are no published reports of the impact of VitE on the primary prevention of cardiovascular disease in humans. This may be more important since oxidative stress-induced injury in diabetes may not, once established, be reversible by later antioxidant therapy [1].

To test this hypothesis, we used the well-characterised rat STZ model to examine the ability of a higher dose of VitE early in the course of diabetes to prevent or reduce endothelial vasodilator dysfunction and changes in mechanical wall properties in arteries. The effects of VitE were studied in two muscular arteries, the mesenteric and femoral arteries. We examined possible mechanisms of action for vitamin E, including direct effects on the level of reactive oxygen species, effects on arterial wall PKC-β_2, and effects on AGE accumulation.

Materials and methods

Animals. Diabetes was induced in 8-week-old male Wistar rats (Central Animal Services, Monash University, Melbourne, Australia) by injection of STZ (60 mg/kg) into the tail vein as described previously [14]. Diabetic rats and control rats received standard chow (containing 50 mg VitE per kg) or chow supplemented with (+)-α-tocopherol (VitE) at 1 g/kg of rat body weight per day. Since diabetes is associated with hyperphagia, diabetic and control rats were caged separately and extra standard chow was provided subsequently to the diabetic animals. After 8 weeks the rats were anaesthetised, weighed and killed by decapitation. The experiments in this study comply with the guidelines of the National Health and Medical Research Council of Australia: "Code of Practice for the Care and Use of Animals for Scientific Purposes".

Blood was collected and plasma glucose concentration was measured immediately. Plasma and whole blood was frozen (−80 °C) for later measurements of glycohaemoglobin (GHb), lipids, plasma VitE and 8-isoprostane concentrations. Femoral arteries and the mesenteric bed were freed of adipose and loose connective tissue. Lengths of 10 to 15 mm of each bed were retained for immediate study while the remaining tissue was weighed and snap-frozen in liquid nitrogen for localisation and semi-quantification of arterial wall PKC-β_2.

Functional studies. Segments of 1° femoral artery and 2° mesenteric arcade were mounted on a myograph for measurement of isometric tension [14]. Segments were continuously superfused with isoglycaemic physiological saline solution (PSS) at 35 °C at 4 ml/min. The solution contained (mmol/l): NaCl 120, KCl 5, NaHCO$_3$ 25, KH$_2$PO$_4$ 1, MgSO$_4$ 1.2, CaCl$_2$ 2.5, glucose 11, and was gassed with O$_2$ 95%, CO$_2$ 5%. Endothelial function was tested using acetylcholine (ACh) in segments pre-contracted with phenylephrine (10^{-6}–10^{-5} mol/l). Concentration-relaxation curves were obtained in control PSS, in N$^\omega$-nitro-L-arginine methyl ester (L-NAME, 10^{-4} mol/l), and L-NAME and indomethacin (10^{-6} mol/l) as described [14]. Relaxations persisting in the presence of both blockers represent the contribution of endothelium-derived hyperpolarising factor (EDHF) [14]. Endothelium-independent relaxation was tested using sodium nitroprusside (SNP). All drugs were purchased from Sigma, St Louis, Mo., USA.

Passive mechanical wall properties. Leak-free segments of each artery were mounted on a pressure myograph, the proximal end tied to a glass cannula, the distal end tied off and set pressure maintained via a servo-control system (Living Systems, Burlington, Vt., USA). The myograph was mounted on an inverted microscope fitted with a video camera and the image was projected on to a monitor screen, from which length, wall thickness and outside diameter of the segment were measured (×300 magnification). Segments were superfused at 10 ml/min with zero-Ca^{2+} PSS containing EGTA (1 mmol/l). Intraluminal pressure (P) was increased in increments (10–120 mm Hg, expressed in Pa), and external diameter (OD), length (L) and wall thickness (h) were measured (in µm) at each pressure. The following parameters were derived: internal diameter (ID µm) = OD−2h; wall stress (kPa) = P(ID)/(2h); wall strain = ID−(ID$_{\text{extrapolated to 0 mm Hg pressure}}$)/(ID$_{\text{extrapolated to 0 mm Hg pressure}}$). The rate constant of an exponential function fitted to the stress–strain relationship was determined using Prism software (GraphPad, San Diego, Calif., USA).

AGE determinations. Vascular AGE content was determined by immunohistochemistry as described [15]. In brief, sections were incubated with a polyclonal AGE antibody that recognises the non-fluorescent AGE, carboxymethyllysine [16]. Vascular AGE staining was then characterised using semi-quantitative image analysis methods. The AGE-associated fluorescence of collagen extracted was also estimated in vascular tissue samples following pepsin digestion (400 mg/ml) and acid hydrolysis [17]. Results were normalised for the hydroxyproline content of the supernatant determined by standard methods [18]. Incomplete digestion of AGE-modified tissue proteins results in the production of AGE-peptides with a low molecular weight. These accumulate in the serum and accurately reflect the level of tissue AGE-modification in experimental diabetes [19]. Plasma AGE-peptides present in plasma samples were assayed using a flow injection assay system with on-line spectrofluorometric detection [19].

Protein kinase C-$\beta2$ immunohistochemistry. A modification of the ABC immunoglobulin enzyme bridge technique [20] was used on formalin-fixed paraffin sections of vessels. Endogenous peroxidase activity was eliminated by incubating sections in 0.3% hydrogen peroxide for 20 min. Sections were then incubated with normal goat serum for 20 min and with PKC-β_2 primary antibody (Santa Cruz Biotechnologies, Santa Cruz, Calif., USA). The latter was done overnight at 4 °C. Tissue sections were consecutively stained with biotinylated IgG and avidin-biotin horseradish peroxidase complex (Vectastain ABC ELITE kit, Vector Laboratories, Burlingame, Calif., USA). Peroxidase activity was demonstrated using 3,3-diaminobenzidine in 0.03% hydrogen peroxide. Sections were counterstained in Harris' haematoxylin and mounted in dePex (BDH, Merck, Poole, UK).

Blood glucose and GHb levels. Blood glucose (mmol/l) was measured by reflectance as described [14]. GHb levels were

Table 1. Body weights and biochemical parameters

	Control rats		Diabetic rats	
	Std chow	VitE	Std chow	VitE
Number (n)	14	19	12	17
Body weight (g)	486±10	460±10	309±14[a]	306±16[a]
Glucose (mmol/l)	7.4±0.3	8.3±0.5	36.0±1.7[a]	33.7±1.5[a]
GHb (%)	4.2±0.1	4.0±0.1	15.8±0.5[a]	14.9±0.5[a]
Total cholesterol (mmol/l)	1.62±0.06	1.32±0.04[b]	1.99±0.09[a]	2.04±0.10[a]
Triglycerides (mmol/l)	1.76±0.11	1.34±0.10[b]	2.21±0.34	4.14±0.55[a,b]
HDL-cholesterol (mmol/l)	0.75±0.02	0.64±0.02[b]	1.05±0.05[a]	1.01±0.04[a]
Isoprostanes (pg/ml)	15.2±3.3	6.4±2.0[b]	33.3±4.4[a]	27.8±3.6[a]

Values are means ± SEM after 8 weeks of treatment with standard chow (Std chow, no vitamin E added) or vitamin E supplementation (VitE) in control and diabetic rats. [a] $p<0.05$ for difference diabetic vs control rats; [b] $p<0.05$ for difference between VitE rats and Std chow rats. GHb, glycohaemoglobin

measured by an automated HPLC assay based on affinity chromatography (CLC330 HbA_1c analyser; Primus, Kansas City, Kan., USA).

Lipid measurements. Total cholesterol, triglycerides and HDL-cholesterol were measured by commercial enzymatic assays using a DADE Behring Dimension RxL Chemistry Analyser (Dade Behring Diagnostics, Sydney, Australia). CVs were 1.9% at 3.4 mmol/l, 1.3% at 7 mmol/l and 4.4% at 0.65 mmol/l.

Plasma vitamin E concentrations. Plasma vitamin E (α and γ tocopherol) was measured using reverse phase HPLC with an ultraviolet absorption detector [21].

Plasma isoprostane. Free plasma 8-isoprostane was analysed by an Enzymatic Immuno Assay (Cayman Chemical, Ann Arbor, Mich., USA). The performance characteristics as assessed by the manufacturer for intra- and inter-assay CVs were less than 10%.

Data analysis. Relaxations (tension) were expressed as per cent of the contraction evoked by phenylephrine. Sigmoid curves were fitted to concentration–relaxation curves using the least-squares method (Prism software) [14]. Maximal relaxation (V_{max}), the concentration of drug that produced 50% of V_{max} (EC_{50}) and pD_2 values ($-\log EC_{50}$) were compared between treatment groups. Values of n quoted indicate the number of rats in the group. Student's t test or ANOVA were used for testing, and differences were accepted as statistically significant when p was less than 0.05.

Results

Animal weights and circulating biochemical parameters

Body weight. Body weights of control (250±3 g, n=33) and diabetic rats (256±4 g, n=29) were similar (p=0.3) at the time of STZ injection. Weights at 8 weeks following injection were significantly lower in diabetic animals and were not affected by VitE (Table 1).

Blood glucose and GHb levels. At 8 weeks, diabetic rats were hyperglycaemic and GHb levels were elevated. VitE did not influence these variables (Table 1).

Plasma vitamin E levels. Total plasma vitamin E levels were similar in all rats fed standard chow (control 24±2 [n=14] versus diabetic rats 25±2 μmol/l [n=12]), with most being α-tocopherol. Levels of α-tocopherol were more than three-fold greater in rats receiving VitE (control 66±6 [n=19] vs diabetic rats 196± 40 μmol/l [n=17], p<0.01).

Lipids. Total cholesterol, HDL and triglyceride levels were higher in diabetic than in control rats. VitE was associated with a decrease in all of these parameters in controls, but not with lower plasma cholesterol levels in diabetic rats (Table 1).

Isoprostanes. Plasma total isoprostane concentrations were about two-fold higher in diabetic than in control rats (p<0.01). VitE was associated with a significant reduction in isoprostane in control but not in diabetic rats (Table 1).

Functional responses

Endothelium-dependent vasodilatation in mesenteric arteries. In rats fed standard chow, higher concentrations of ACh were required to achieve relaxation in mesenteric arteries of diabetic compared with control rats (pD_2 7.10±0.08, n=9, vs 7.70±0.04, n=10, p<0.0001). VitE had no effect on ACh-induced relaxation in arteries from controls but significantly improved the sensitivity to ACh (pD_2 7.42±0.04, n=12, p=0.0005) in vessels of diabetic rats (Fig. 1a, b). The pD_2 value in the presence of L-NAME and indomethacin, which represents EDHF, was significantly reduced in diabetic rats (pD_2 6.03±0.21, n=9, vs 6.67±0.07, n=10, p=0.007) and was not affected by VitE in vessels of either control or diabetic rats (Fig. 1a, b).
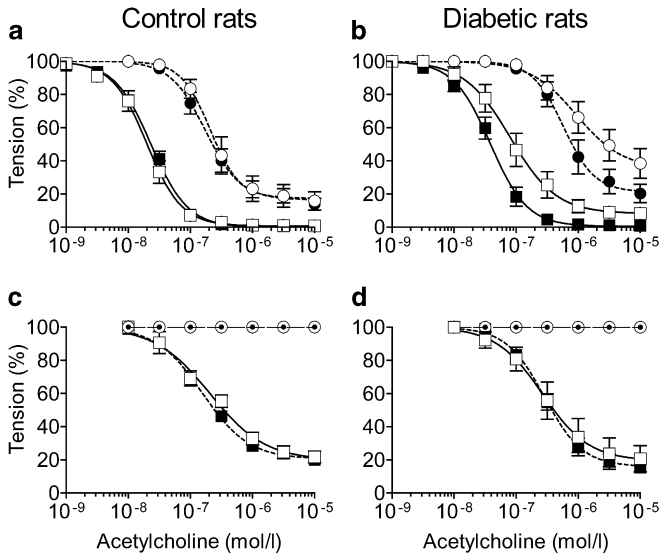
Fig. 1. Acetylcholine reduced the tension evoked by phenylephrine in segments of mesenteric (**a, b**) and femoral (**c, d**) arteries (squares). Subsequently, nitric oxide and prostanoid production were blocked with L-NAME and indomethacin respectively (circles). Rats fed with standard chow, open symbols; rats fed with vitamin E supplement, filled symbols

Table 2. Effect of vitamin E on endothelium-independent relaxation in response to sodium nitroprusside

Mesenteric artery			
Control rats	n	pD_2	V_{max} (%)
No supplementation	8	7.54±0.06	6±3
VitE	13	7.81±0.05[a]	4±3
Diabetic rats			
No supplementation	7	7.39±0.07	12±4
VitE	11	7.81±0.08[a]	3±4
Femoral artery			
Control rats	n	pD_2	V_{max} (%)
No supplementation	6	7.43±0.07	3±3
VitE	7	7.54±0.13	4±6
Diabetic rats			
No supplementation	6	7.39±0.09	4±4
VitE	7	7.47±0.06	8±3

Maximal relaxation (V_{max}) and pD_2 for sodium nitroprusside in mesenteric and femoral arteries from control and diabetic rats fed either standard chow (no supplementation) or standard chow supplemented by vitamin E (VitE). [a] $p<0.05$ for difference between arteries from VitE rats and rats fed standard chow
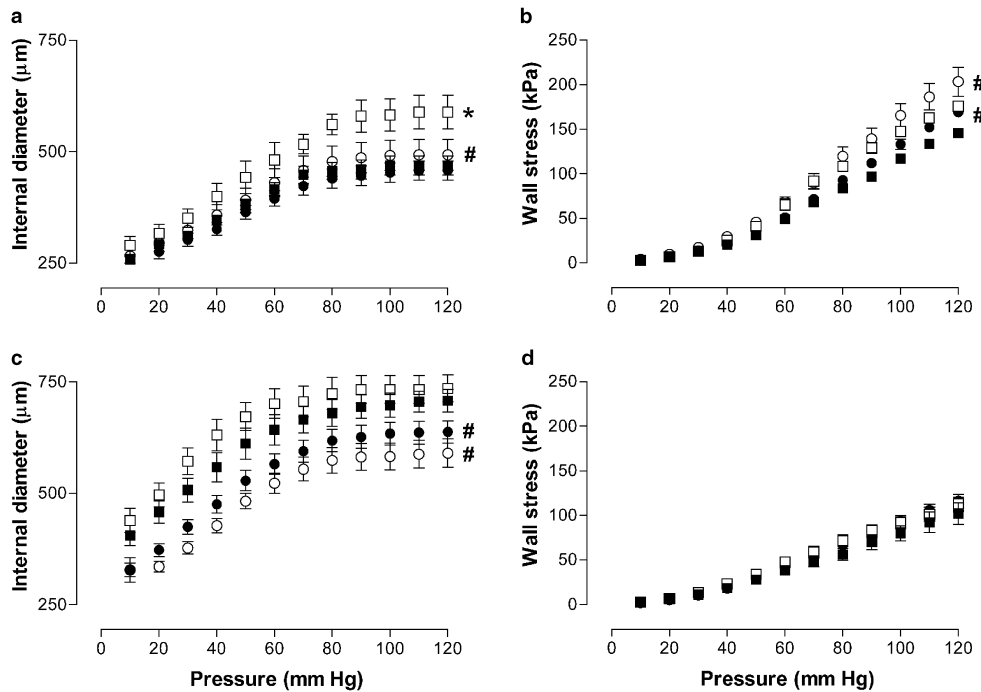

Fig. 2. Internal diameter and wall stress of arterial segments. Segments were superfused in the absence of calcium and pressure was increased incrementally. Internal diameter was measured and wall stress calculated in mesenteric (**a, b**) and femoral (**c, d**) arteries from control (filled symbols) and diabetic rats (open symbols) fed either standard chow (controls $n=4$; diabetic $n=3$) or standard chow with added vitamin E ($n=10$ controls; $n=8$ diabetic). Squares, rats on standard chow; circles, rats on added vitamin E. *, $p<0.05$ control vs diabetic rats; #, $p<0.05$ added vitamin E vs standard chow, for entire curve

Endothelium-dependent vasodilatation in femoral arteries. There was no difference in endothelium-dependent relaxation in femoral arteries of diabetic or control rats fed standard chow or VitE (Fig. 1c, d). Endothelium-dependent relaxation was abolished in all femoral arteries by L-NAME (Fig. 1c, d).

Endothelium-independent vasodilatation. In rats fed standard chow, there was no difference between diabetic and control rats with regard to relaxation of mesenteric and femoral arteries in response to SNP (Table 2). VitE significantly improved the sensitivity
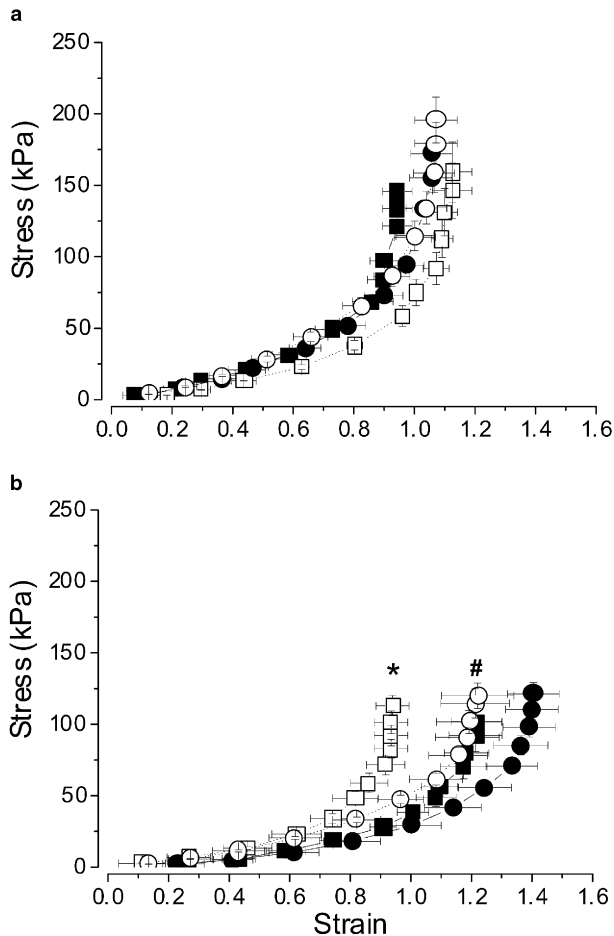
Fig. 3. In mesenteric arteries (**a**), there was no difference in the stress–strain relationship in diabetes or following vitamin E supplementation (VitE). Conversely in femoral arteries (**b**), stiffness was increased in diabetes and this was prevented by VitE. Filled squares: control rats fed standard chow; open squares: diabetic rats fed standard chow. Filled circles: control rats fed VitE; open circles: diabetic rats fed VitE. * $p < 0.05$ control vs diabetic rats. # $p < 0.05$ VitE vs standard chow for the entire curve. Numbers of animals: as Figure 2

Fig. 4. Protein kinase C (PKC)-β_2 was significantly greater in mesenteric arteries of diabetic rats fed normal chow (**c**) than in control rats on standard chow (**a**) and this was normalised by vitamin E supplementation (**d**). Combined data are shown in (**e**). $n=7$ in (**a**) and (**b**); $n=8$ in (**c**) and (**d**); * significantly different from control rats. C+VitE, control rats on vitamin E supplement; D+VitE, diabetic rats on vitamin E supplement

to SNP in mesenteric arteries of diabetic and control rats, but did not affect SNP-induced relaxation in femoral arteries (Table 2). The improvement in sensitivity to endothelium-independent SNP and endothelium-derived nitric oxide (NO) in mesenteric arteries from diabetic rats was equivalent at approximately two-fold in both cases.

Passive mechanical wall properties

Mesenteric arteries. The maximum ID reached was significantly greater in vessels from diabetic than in those from control rats fed standard chow. Wall thickness at 90–120 mm Hg remained the same (diabetic, 27±2 μm, $n=3$ vs control rats, 26±2 μm, $n=4$). Wall stress was increased in diabetic arteries (Fig. 2), reflecting the difference in internal diameter. The rate constant of the stress–strain relationship was similar
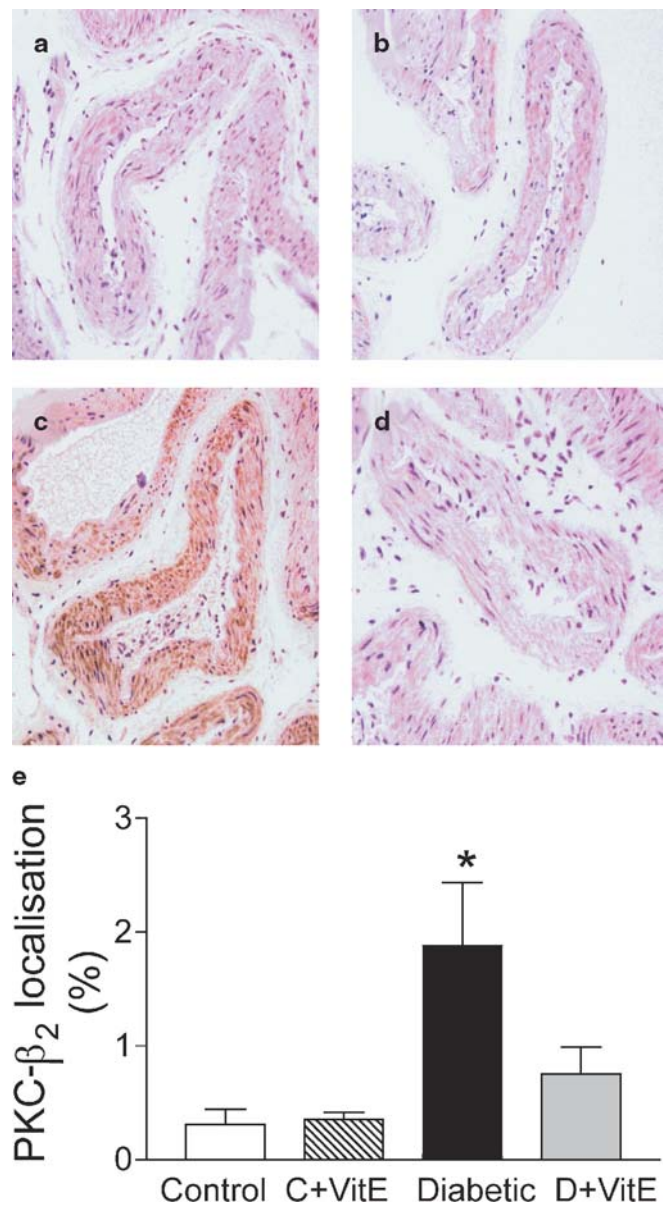
in vessels from control (4.85±0.77, $n=4$) and diabetic rats (5.11±0.62, $n=3$) (Fig. 3).

VitE resulted in a striking thinning of the walls of mesenteric arteries from both control (21±1 μm, $n=10$) and diabetic rats (21±1 μm, $n=8$), and prevented the increase in internal diameter in diabetic rats (Fig. 2). Wall stress was significantly increased by VitE in tissues from control and diabetic rats (Fig. 2), reflecting the reduced wall thickness. The rate constant of the stress–strain relationship in arteries from control (5.03±0.65, $n=10$) and diabetic rats (5.40±0.77, $n=8$) was unchanged following VitE (Fig. 3).
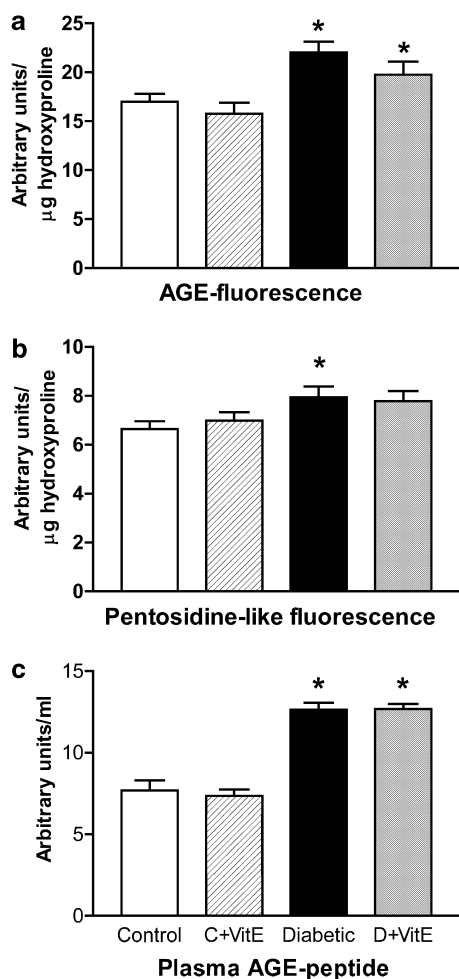
Fig. 5. AGE and pentosidine-like fluorescence in mesenteric arteries and plasma AGE peptide were all increased in diabetic rats fed normal chow and were not influenced by vitamin E supplementation (VitE). * $p<0.05$ control vs diabetic rats; $n=6$ (**a, b**); (**c**) $n=13$ control, $n=19$ C+VitE (control + VitE), $n=12$ diabetic, $n=16$ D+VitE (diabetic + VitE)

Femoral arteries. Internal diameter, wall thickness at 90–120 mm Hg (diabetic, 52±1 μm, $n=3$ vs control rats, 58±6 μm, $n=4$) and wall stress were similar in femoral arteries from diabetic and control rats (Fig. 2). VitE significantly reduced wall thickness (diabetic, 39±2 μm, $n=8$ vs control rats, 44±3 μm, $n=10$) but wall stress was not affected (Fig. 2). The stress–strain relationship was markedly shifted to the left in femoral arteries from diabetic vs control rats, with a significantly elevated rate constant (diabetic 6.27±0.76 vs control rats 4.11±0.32, $p=0.03$), and this was prevented by VitE (diabetic, 4.15±0.65, $n=8$ vs control rats, 4.57±0.77, $n=10$) (Fig. 3).

Protein kinase C-β_2 localisation and levels

Levels of the PKC isoform PKC-β_2, as assessed by immunohistochemical staining, were greater in mesenteric arteries from diabetic ($n=8$) (Fig. 4c, e) than in those from control rats fed standard chow ($n=7$; $p=0.03$) (Fig. 4a, e). VitE did not affect PKC-β_2 levels in mesenteric arteries from control rats ($n=7$; $p=0.8$) (Fig. 4b, e), but in arteries from diabetic rats the levels were reduced to values that did not differ from controls ($n=8$; $p=0.2$) (Fig. 4d, e).

Levels of PKC-β_2 were also higher in femoral arteries of diabetic (3.8±0.8%, $n=8$) vs control rats (1.3±0.4%, $n=8$, $p=0.02$) fed standard chow, but were unchanged by VitE (diabetic, 2.6±0.7%, $n=10$, $p=0.3$ vs control rats, 0.7±0.1%, $n=9$, $p=0.2$).

In both arteries, immunohistochemical staining appeared to be greatest in the smooth muscle cells of the medial layer.

AGE localisation and levels

Vascular staining for AGE was significantly increased in vessels of diabetic versus control rats, both in mesenteric arteries (diabetic, 4.0±0.3%, $n=5$ vs control rats, 2.2±0.6%, $n=5$, $p<0.01$) and femoral arteries (diabetic, 3.6±0.4%, $n=6$ vs control rats, 0.9±0.2%, $n=7$, $p<0.01$). Mesenteric artery AGE-fluorescence and plasma AGE peptide were also increased in diabetic rats to a similar extent (Fig. 5). VitE did not significantly modify vascular AGE-staining or AGE-fluorescence in either rat group.

Discussion

This study demonstrates that early VitE improves endothelium-dependent relaxation in small resistance mesenteric arteries of diabetic rats (Table 3). This is consistent with observations in patients with diabetes [10]. Some previous studies of VitE in STZ-induced diabetes have failed to show any significant effect on endothelium-dependent relaxation in the mesenteric arteries, although in those studies a five- to ten-fold lower dose of VitE was used [9]. Indeed, supplementation with a higher dose of VitE improved endothelium-dependent relaxation in the aorta [8] and prevented the decline in blood flow to hindlimb nerves after 2 months of STZ-induced diabetes [22], similar to our present findings in small resistance arteries.

The effect of early VitE on vasorelaxation in diabetes appears to reflect an improvement in the response to NO, as the component of endothelium-dependent relaxation attributed to EDHF was unaltered. Significantly, VitE enhanced endothelium-independent relaxation in response to SNP in mesenteric arteries, indicating a direct effect of VitE on NO availability and/or vasodilator mechanisms in the smooth muscle (Table 3). VitE improved the component of endothelium-dependent vasodilation attributable to NO in mesenteric arteries, although this component did not appear to be impaired by diabetes. In contrast, VitE had little

Table 3. Summary of the effects of diabetes and vitamin E supplementation in diabetes on endothelium-dependent and -independent vasodilation, passive wall properties and plasma PKC-β levels in mesenteric and femoral arteries

	Endo-dependent		Endo-independent		Passive wall properties		PKC-β levels	
	MA	FA	MA	FA	MA	FA	MA	FA
Diabetes	↓	↔	↔	↔	↑ diam.	Stiffer	↑	↑
VitE in diabetes	Rest.	↔	↑	↔	Rest.	Rest.	Rest.	↔

Effects shown are for mesenteric arteries (MA) and femoral arteries (FA) of diabetic vs control rats. ↑, increased; ↓, decreased; ↔ not different from control; diam., diameter; Rest., restoration of control values; VitE, chow supplemented with vitamin E

effect on the response attributable to EDHF, the principal component of vasodilation impaired in diabetes.

Improvements in vasodilator function in small mesenteric arteries in this study were independent of changes in plasma isoprostanes. Important physiological effects of VitE involve its incorporation into vascular tissue [23], including inhibition of leucocyte adhesion to endothelial cells [24], reduced smooth muscle proliferation [25] and protection against oxidant-mediated cytotoxicity [26]. It is possible that the benefit derived from VitE on endothelial function may be concentration-dependent and occur regardless of its effect on oxidant stress as measured by isoprostane levels. Nevertheless, isoprostane levels cannot be considered universal markers of oxidative stress, in particular in the vasculature where other pathways may predominate, such as NADPH oxidase [27].

Diabetes is associated with markedly increased expression of PKC-β, especially in the smooth muscle layer (Fig. 4). Early VitE prevented the diabetes-associated rise in PKC-β levels in mesenteric arteries of diabetic rats (Table 3). PKC-β has significant actions in endothelial and smooth muscle cells of blood vessels [1, 28]. PKC may contribute to abnormal vascular reactivity via increased production of superoxide radicals [20], and in endothelial cells it may impair NO production by reducing NO synthase protein levels [29]. In vascular smooth muscle, enhanced PKC-β_2 activity is associated with hypertrophy and hyperplasia [4]. Enhanced plasma membrane phospholipase D activity [4] and an increase in diacylglycerol levels [30] are thought to underlie the enhanced PKC-β_2 activity in hyperglycaemia. The inhibition of PKC-β_2 activation by VitE has been documented in diabetes [4, 6, 31]. VitE abrogates the increase in hyperglycaemia-induced PKC-β_2, diacylglycerol, phospholipase D and changes in cell number and size. The mechanisms by which VitE inhibits PKC activation are unclear. The fact that other antioxidants, even β-tocopherol, do not inhibit PKC suggests that these mechanisms are not related to the anti-oxidant effect of vitamin E [32]. The inhibitory effect of α-tocopherol (VitE) on PKC activation is concordant with its effect on phosphorylation of the myristolated, alanine-rich C kinase substrate protein, a substrate of PKC [25], and with the fact that it also increased protein

phosphatase type 2A activity, which results in PKC-dephosphorylation [32]. These observations suggest that a ligand/receptor type of mechanism is the basis of the action of VitE on PKC. There is also evidence that VitE may inhibit PKC translocation to the cell membrane [25].

VitE had no effect on endothelium-dependent relaxation in femoral arteries from diabetic rats (Table 3). We have previously reported that this artery (at ~300 μm diameter) is resistant to the effects of diabetes [14]. In contrast to the mesenteric arteries, the significantly elevated PKC-β_2 activation in femoral arteries from diabetic rats was not affected by VitE (Table 3). This suggests that PKC-β_2 activation does not have deleterious effects on relaxation pathways in this artery and may explain its resistance to the effects of diabetes. It may be that metabolic cross-talk between the endothelium and smooth muscle is required for the expression of dysfunction involving PKC-β. This notion arises from our previous observations showing that, while endothelial and smooth muscle cells are well connected via myo-endothelial gap junctions in mesenteric arteries, the two cell types are not connected in femoral arteries [33]. This difference may provide a clue to resolving the different susceptibilities between arterial beds to dysfunction in diabetes, a suggestion that requires further study.

There are no previous studies of the effect of VitE on passive mechanical wall properties of resistance vessels. Previous studies in patients with Type 1 diabetes failed to demonstrate any significant effect of VitE on brachial arterial compliance measured in vivo [10, 34], despite improved endothelium-dependent vasodilatation. Increased intestinal perfusion in the setting of diabetes-induced hyperphagia may contribute to an increase in maximum internal diameter in mesenteric arteries (Table 3). The fact that this increase in diameter was not associated with a change in the stress–strain relationship suggests remodelling in the absence of a change in mechanical properties. The relatively small number of control tissues studied means that these conclusions are made with caution.

The similarity between vascular flow responses of pair-fed diabetic rats and those of diabetic rats with free access to food suggests that diabetes, and not hyperphagia, may determine the changes mentioned

above [35]. In contrast, femoral arteries appeared to be restricted in their ability to increase in diameter and were significantly stiffer in diabetes (Table 3), with the suggestion of a change in wall composition. While VitE markedly reduced wall thickness in both arteries, this is probably achieved by re-arrangement of the components and/or structure of the wall, rather than by an altered wall composition in mesenteric arteries. Again, the number of control tissues here was relatively small. VitE prevented the diabetes-associated stiffening in femoral arteries. This is likely to be independent of PKC-β since the content of this kinase in femoral arteries was unchanged by VitE. While AGE are important causes of altered mechanical wall properties in diabetes [5, 36], VitE did not influence the AGE content of arteries from diabetic rats.

A number of studies, primarily in Type 2 diabetes, have assessed the role of VitE and related compounds on endothelial function and oxidative stress, with varying results [37, 38]. Nevertheless, recent large trials [12, 13] have reported no benefit from VitE in the prevention of cardiovascular disease in individuals with and without diabetes. It should be appreciated that the data presented here suggest that the potential benefit of VitE in diabetes occurs early, prior to the onset of irreversible structural and functional changes in resistance vessels. As a result, VitE may be better used in the primary prevention of vascular disease, rather than the secondary prevention of vascular events in patients with established vascular disease.

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