# Antioxidant and Triglyceride-Lowering Effects of Vitamin E Associated With the Prevention of Abnormalities in the Reactivity and Morphology of Aorta From Streptozotocin-Diabetic Rats

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**In this study, we evaluated the effects of vitamin E on the vascular reactivity and structure of thoracic aorta from streptozotocin (STZ)-diabetic rats. Plasma glucose, cholesterol, and triglyceride concentrations in rats were increased markedly by STZ-diabetes. The thiobarbituric acid (TBA) reactivity level as an index of lipid peroxidation was higher in both plasma and aorta of STZ-diabetic rats compared with controls. The rings of thoracic aorta with or without endothelium were mounted in organ chambers for measurement of isometric tension and were contracted by a single dose ($10^{-5}$ mol/L) and then cumulative doses of noradrenaline ([NA] $10^{-9}$ to $10^{-5}$ mol/L). Pretreatment with methylene blue (MB) or removal of the endothelium resulted in a similar degree of enhancement in NA-induced contraction of control rings. STZ-diabetes increased the fast and slow components of NA-induced contraction in all experiments. The maximal contractile response of aorta to NA was also augmented by STZ-diabetes, whereas the sensitivity ($pD_2$) remained unaltered. STZ-diabetes resulted in significant increases in the maximum contractile response and sensitivity of aorta to KCl. STZ-diabetic rats showed a significant reduction in the percentage of endothelial response (PER). A group of diabetic rats was treated from the time of diabetes induction with a 0.5% dietary supplement of vitamin E. Vitamin E supplementation of STZ-diabetic rats eliminated accumulation of lipid peroxides and returned plasma triglycerides toward normal levels. Diabetes-induced abnormal contractility and endothelial dysfunction were significantly but not completely prevented by vitamin E treatment. The endothelium-independent relaxation response to sodium nitroprusside (SNP) was not affected by diabetes or vitamin E treatment. Electron microscopic examination of thoracic aorta revealed that normal tissue organization was disrupted in STZ-diabetic rats, and that vitamin E treatment can protect the morphological integrity of aorta against STZ-diabetes. The results suggest the following: (1) The increased triglycerides/lipid peroxides may be an important reason for morphological or functional disruption of endothelium and enhanced activation of contractile mechanisms of vascular smooth muscle in STZ-diabetic rats. Both contribute to an increased responsiveness of diabetic aorta to vasoconstrictor agents. (2) Vitamin E treatment of STZ-diabetic rats can prevent the development of abnormal contractility and structure and endothelial dysfunction in aorta. (3) The triglyceride- and/or lipid peroxidation–lowering effect of vitamin E may be crucial for the protective effect of this vitamin on the vasculature.**


IT IS NOW CLEAR that impaired glucose metabolism leads to oxidative stress and that glycation of proteins produces free oxygen radicals.[1] In diabetes, oxidative stress has been found to be mainly due to an increased production of free radicals and a sharp reduction of antioxidant defenses.[1,2] The relationship between oxidative stress and diabetic complications has been extensively investigated. Briefly, oxidative stress has been suggested to be a major factor in the genesis of both macroangiopathy and microangiopathy in diabetes.[3-5] The hyperreactivity in the contractile response to vasoconstricting agents together with a deficiency in the vasorelaxant capacity of endothelium has been discussed as being a result of oxidative stress in diabetic animals.[5,6] Vitamin E, a membrane-bound, lipid-soluble, and naturally occurring antioxidant,[7] has been shown to protect animal tissues against oxidative damage such as lipid peroxidation both in vitro[8] and in vivo.[9] In addition to its role in the protection of biological membranes against injury induced by free radicals, there is now strong evidence suggesting that vitamin E can modulate a variety of endothelial cell functions such as prostacyclin synthesis,[10] endothelium-mediated relaxation,[11] and cell growth and/or repair.[12] Moreover, vitamin E can restore the impaired prostacyclin/thromboxane $A_2$ balance in diabetic rats,[13] and reverses the increased protein kinase C (PKC) activity in hyperglycemic aorta.[14,15] It has been reported that vitamin E content in tissues is decreased in diabetic animals,[16] and a high concentration of glucose can reduce D-α-tocopherol binding in tissues.[17] We have previously found that vitamin E treatment is beneficial to the protection of motor and sensory nerve conduction against STZ-diabetes–induced injury, and that this vitamin can prevent abnormalities in nerve blood flow if it is added to the diet for a period of 4 to 5 weeks.[18] Similarly, it has been demonstrated that a 0.1%[19] or 0.2%[20,21] vitamin E diet has a beneficial effect on the protection of endothelial function in hypercholesterolemic rabbits. In a recent study by Keegan et al,[22] it has also been reported that the defective endothelium-dependent relaxation of aorta from STZ-diabetic rats is prevented by 1% dietary supplementation of vitamin E. However, the exact mechanism(s) by which vitamin E prevents endothelial dysfunction is still unknown. Accordingly, in this study we examined the contractile effects of noradrenaline (NA) and KCl in aortic rings with or without endothelium from normal and STZ-diabetic rats, and investigated the protective role and its possible mechanism(s) of vitamin E treatment on the vasculature.

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## MATERIALS AND METHODS

### *Experimental Organization*

Male albino rats (8 weeks old, 200 to 250 g) were divided randomly into three experimental groups. Nondiabetic rats were used as a control group (C group). Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg freshly dissolved in sterile 0.9% aqueous NaCl). Two days later, diabetes was verified by estimating hyperglycemia on tail vein blood (Ames Glucometer; Miles Laboratories, Elkhart, IN). Rats who had blood glucose levels of 15 mmol/L or greater were considered diabetic. One group of diabetic rats remained untreated (D group). Control and untreated diabetic rats were maintained on a standard laboratory chow diet. The other group of diabetic rats (E group) was placed on a high–vitamin E supplemented diet. To obtain a high–vitamin E diet, the standard laboratory chow diet was supplemented with tocopheryl acetate (DL-α-tocopheryl acetate as Ephynal; Roche, Istanbul, Turkey) to increase the amount of vitamin E to 5 g DL-α-tocopheryl acetate/kg, corresponding to 7,450 IU/kg diet. All groups of rats were maintained under standard housing conditions for a period of 8 to 9 weeks with free access to food and water.

### *Preparation of Tissues*

Food was withdrawn for 18 hours before killing the rats. Rats were anesthetized with diethyl ether and injected with heparin sodium (200 U intravenously). Before the aortae were removed, a sample of blood was obtained by cardiac puncture for analytical procedures. The blood samples were immediately centrifuged at 3,000 rpm for 20 minutes, and the obtained plasma samples were stored at −25°C until analyzed. Thoracic aortae were quickly excised and placed in a petri dish filled with Krebs solution with the following composition (in mmol/L): NaCl 118.5, KCl 4.74, $CaCl_2$ 2.5, $MgSO_4$ 1.18, $KH_2PO_4$ 1.18, $NaHCO_3$ 24.9, and glucose 10.0. Rings approximately 3 to 4 mm in length were cut. One ring of each pair was left intact; in the other ring, endothelium was mechanically removed. Rings with or without endothelium were mounted horizontally by a pair of stainless steel hooks in tissue baths to measure tension. Tissue baths were filled with 20 mL Krebs solution continuously bubbled with a mixture of 5% $CO_2$:95% $O_2$, pH 7.4, at 37°C. Tension was measured by an isometric force transducer (no. 7004; Ugo Basile, Varese, Italy) connected to a microdynamometer (Unirecord; Ugo Basile).

### *Experimental Protocol*

Preparations were allowed to equilibrate for 90 minutes under a resting tension of 2 g. During the equilibration period, the solution in the tissue bath was replaced every 30 minutes. Successful removal of the endothelium was confirmed by loss of acetylcholine ($10^{-5}$ mol/L)-induced relaxation in preconstricted rings by NA ($10^{-6}$ mol/L).

At the end of the equilibration period, dose-response curves were obtained with NA and then with KCl. NA or KCl was added in a cumulative manner until a maximal response was achieved. After addition of each dose, a plateau response was obtained before addition of a subsequent dose. Before starting the dose-response curves with NA, the aortic rings with or without endothelium were exposed to $10^{-5}$ mol/L NA until the contraction reached a plateau, to measure the fast and slow components of the vascular response to NA. Some rings with endothelium were incubated with methylene blue (MB) $10^{-6}$ mol/L for 30 minutes before addition of NA.

To evaluate sodium nitroprusside (SNP)-induced vasodilatation, rings with endothelium were preconstricted to their $EC_{80}$ value with NA to obtain a stable plateau, and then the cumulative dose-response curve to SNP was obtained. Consecutive dose-response curves were taken at minimum 30-minute intervals, during which the Krebs solution was changed at least three times.

### *Histology*

For ultrastructural purposes, aortae immediately isolated from the C group (n = 3), D group (n = 3), and E group (n = 3) were cut into small pieces, fixed at 4°C for 2 hours in 2.5% buffered glutaraldehyde, and postfixed for 1.5 hours with 1% osmium tetraoxide. Tissues were dehydrated in ethyl alcohol followed by propylene oxide and embedded in araldite. Ultrathin circumferential sections (at least five sections were taken from three different levels of each specimen) were stained with uranyl acetate and lead citrate and subsequently examined using a Carl-Zeiss electron microscope (Oberkochen, Germany).

### *Biochemical Measurements*

Serum triglyceride, cholesterol, and glucose concentrations were measured on an automatic analyzer (Technicon Technical Instruments, Raritan, NJ) using a commercial kit available from Wako (Wako Chemicals, Osaka, Japan).

*Thiobarbituric acid reactivity.* Malondialdehyde (MDA), an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored complex.[23,24] Measurement of MDA content by TBA reactivity is the most widely used method for assessing lipid peroxidation.[23-25]

TBA reactivity levels in plasma samples of rats were measured according to a slightly modified Satoh method.[24] To 0.5 mL plasma, 2.5 mL 20% trichloroacetic acid (TCA) and 0.025 mL butylated hydroxytoluene ([BHT] 88 mg/10 mL absolute alcohol) were added, and then the tubes were left to stand for 10 minutes at room temperature. After centrifugation, the supernatant was decanted and the precipitate was washed with sulfuric acid. Then, 0.05 mol/L sulfuric acid and 0.8% TBA in 2 mol/L sodium sulfate were added to this precipitate and heated for 30 minutes in a boiling water bath. After cooling, *n*-butanol was added, the tubes were vortexed and centrifuged, and absorbance of the organic phase was read at 530 nm.

TBA reactivity was also measured in aortae of rats according to a slightly modified method.[23] For this purpose, Tris-KCl buffer and BHT were added to tissue homogenates and the mixtures were incubated for 30 minutes at 37°C. Incubations were terminated by addition of TCA and TBA. The tubes were heated for 10 minutes in a boiling water bath. After cooling, pyridine/*n*-butanol and NaOH were added and mixed, and absorbance was read at 548 nm. The results were expressed in terms of MDA equivalents. Freshly prepared MDA tetramethyl acetal solution was used as a standard. BHT, an antioxidant, was added to prevent MDA formation during the assay. Addition of BHT to standard MDA did not affect the color development with TBA.

### *Drugs*

The following pharmacological agents were used: acetylcholine, BHT, NA, MB, MDA tetramethyl acetal, SNP, and STZ (Sigma Chemical, St Louis, MO), KCl (Baker, Phillipsburg, NJ), and DL-α-tocopheryl acetate (a gift from Roche, Istanbul, Turkey).

### *Data Analysis*

The contractile response of aortic rings is expressed as grams of tension per milligram of tissue. The fast component of the NA response was measured from baseline to the point at which the rate of contraction started to decrease abruptly; the slow component was measured from this point to the top of the contraction.[24] The total response was the sum of these two components.[26] Rubyani et al[27] used the difference between the corresponding values for NA-induced contraction in aortae with or without endothelium, the endothelial response (ER), was used to evaluate the suppressing effect of endothelium on NA-induced contraction.[27] In this study, to determine endothelial function, we calculated the

percentage of ER (PER) with the formula, PER = (100 × ER)/total response of aorta to $10^{-5}$ mmol/NA (g tension/mg tissue).

The agonist $pD_2$ value (apparent agonist affinity constant, −log $ED_{50}$) was calculated from each agonist dose-response curve by linear regression analysis of the linear portion of the curve and taken as a measure of the sensitivity of the tissues to each agonist. $ED_{80}$ values were calculated from the cumulative doses of NA that produced 80% of its maximal response for each aorta preparation with endothelium. All values are expressed as the mean ± SEM. The relaxation response to SNP was expressed as a percentage of the precontraction induced by NA. Statistical differences were evaluated using one-way ANOVA followed by the Newman-Keuls test. Statistical significance was indicated at $P$ less than .05 and $P$ less than .01.

## RESULTS

### *Body Weight, Plasma Glucose, Cholesterol, and Triglyceride Concentrations, and TBA Reactivity in Plasma and Aorta*

All diabetic animals showed persistent hyperphagia, polydipsia, and polyuria, which were not modified by vitamin E treatment. Both the D group and E group exhibited hyperglycemia and loss of body weight compared with the C group (Table 1). Plasma cholesterol and triglyceride concentrations markedly increased in the D group. Vitamin E treatment of diabetes did not change plasma glucose or cholesterol concentrations, but significantly prevented ($P < .01$) the accumulation of plasma triglycerides (Table 1). TBA reactivity levels were elevated approximately threefold in plasma and twofold in aorta by diabetes compared with controls, but dietary vitamin E prevented ($P < .01$) the lipid peroxidation significantly (Table 1).

### *Vascular Reactivity*

Cumulative dose-response curves to NA ($10^{-9}$ to $10^{-5}$ mol/L) from aortic rings are shown in Fig 1A. The maximum

**Table 1. Characteristics of Control and Vitamin E–Treated or Untreated Diabetic Rats**

| Characteristic | Control (n = 9) | Untreated Diabetic (n = 8) | Vitamin E–Treated Diabetic (n = 8) |
|---|---|---|---|
| Final body weight (g) | 412 ± 13.0$^{x}$ | 276 ± 11.0$^{y}$ | 298 ± 15.0$^{y}$ |
| Plasma glucose (mmol/L) | 8.2 ± 1.8$^{x}$ | 44.8 ± 4.1$^{y}$ | 42.3 ± 2.8$^{y}$ |
| Plasma cholesterol (mmol/L) | 1.49 ± 0.15$^{x}$ | 4.82 ± 1.31$^{y}$ | 4.71 ± 1.76$^{y}$ |
| Plasma triglyceride (mmol/L) | 1.12 ± 0.21$^{x,X}$ | 3.29 ± 0.31$^{y}$ | 1.65 ± 0.14$^{x,Y}$ |
| TBA reactivity in plasma (nmol MDA/μmol triglyceride) | 0.64 ± 0.02$^{x,X}$ | 1.69 ± 0.08$^{y}$ | 0.95 ± 0.03$^{x,Y}$ |
| TBA reactivity in aorta (nmol MDA/g tissue wet weight) | 170.4 ± 9.5$^{x,X}$ | 334.9 ± 11.6$^{y}$ | 225.8 ± 17.7$^{x,Y}$ |

NOTE. Data (mean ± SEM) were analyzed by ANOVA, and between-group differences for each variable were tested using the Newman-Keuls test: $P < .05$ (X v Y) and $P < .01$ (x v y).

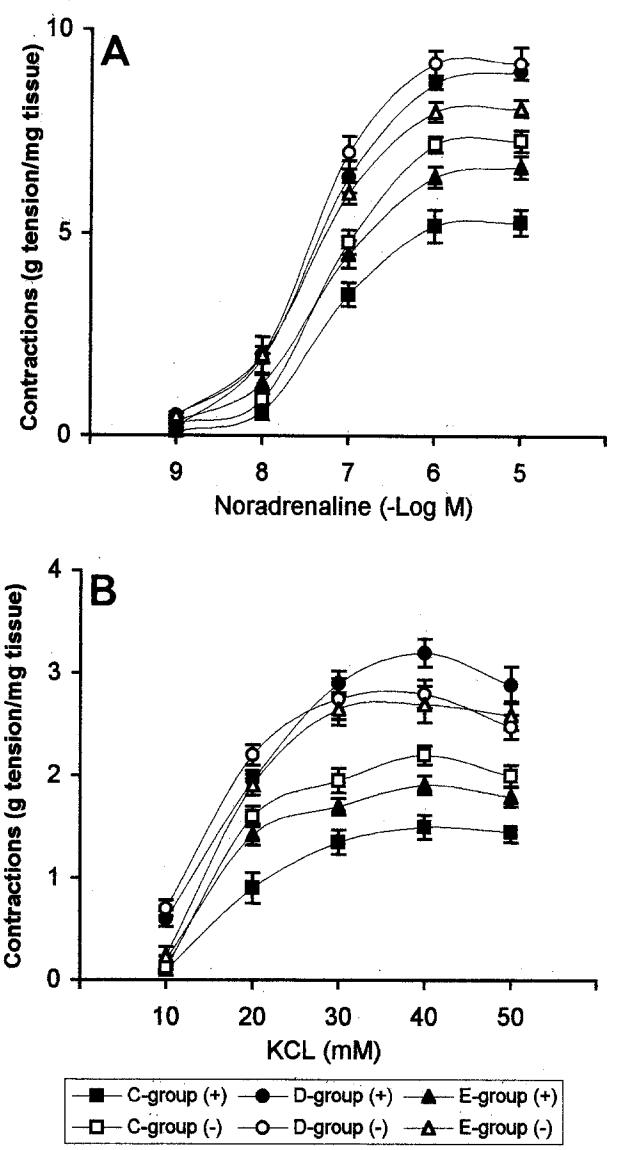


**Fig 1. Cumulative concentration-response curves for NA (A) and KCl (B) in aorta with (+) or without (−) endothelium from control (C group), untreated diabetic (D group), or vitamin E–treated diabetic (E group) rats.**

contractile response to NA was increased significantly in rings with or without endothelium from untreated diabetic rats compared with controls. Removal of the endothelium resulted in a significant increase in the maximum response of aorta in control but not in untreated diabetic rats, indicating that the suppressing effect of endothelium on NA-induced contraction is impaired by diabetes. The $pD_2$ values for aortae to NA were not significantly different in all groups of rats (Table 2). The maximum contractions and sensitivity ($pD_2$) of aortae to KCl increased markedly in untreated diabetic rats compared with controls (Fig 1B and Table 2).

Treatment with vitamin E significantly but not completely prevented these abnormalities in the vascular reactivity of aortae to NA and KCl (Fig 1A and B). In control rats, only the slow component of NA-induced contraction was significantly more pronounced in rings without endothelium than in prepara-

**Table 2. $pD_2$ Values for NA and KCl in Aortae From Control, Untreated Diabetic, and Vitamin E–Treated Diabetic Rats in the Presence (+) or Absence (−) of Endothelium**

| Group | Endothelium | NA | KCL |
|---|---|---|---|
| Control (n = 9) | + | 7.19 ± 0.03 | 1.66 ± 0.01$^{x}$ |
| | − | 7.36 ± 0.01 | 1.71 ± 0.01$^{X}$ |
| Untreated diabetic | + | 7.45 ± 0.02 | 1.82 ± 0.05$^{y}$ |
| (n = 8) | − | 7.47 ± 0.04 | 1.88 ± 0.06b$^{v,Y}$ |
| Vitamin E–treated | + | 7.24 ± 0.05 | 1.76 ± 0.02$^{y}$ |
| diabetic (n = 8) | − | 7.42 ± 0.03 | 1.74 ± 0.01$^{y}$ |

NOTE. Values are expressed as the mean ± SEM. Statistical comparisons are by 1-way ANOVA with the Newman-Keuls test: $P < .05$ (X *v* Y and x *v* y).

tions with endothelium (Fig 2). In all experimental conditions (such as intact endothelium, removed endothelium, or after incubation with MB), the fast and slow responses of aortae to NA in untreated diabetic rats were significantly higher than the responses in control rats (Fig 2). PER was calculated as 58.7% ± 1.1% in control aortae. This was reduced to 11.7% ± 2.3% after 8 weeks of diabetes ($P < .01$). The main PER value determined from vitamin E–treated diabetic rats was 45.6% ± 0.9%, significantly different from the values for untreated diabetic ($P < .01$) or control ($P < .05$) groups. Dose-response curves (Fig 3) and $pD_2$ values for SNP were similar in aortae from the three groups of rats ($pD_2$ values for SNP in control, untreated diabetic, and vitamin E–treated diabetic rats were 7.98 ± 0.01, 8.01 ± 0.02, and 7.94 ± 0.04, respectively).

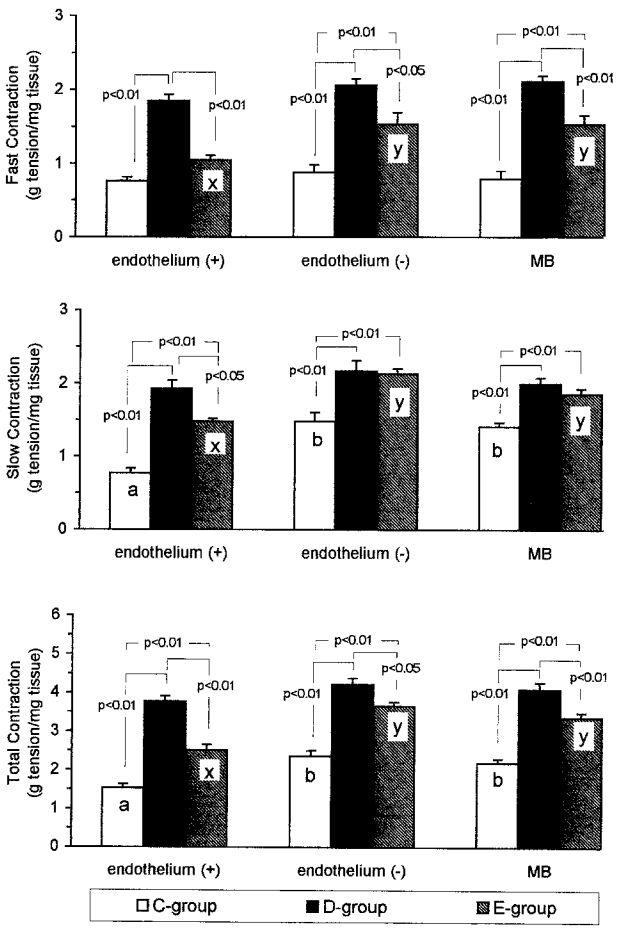


**Fig 2. Fast response, slow response, and total response of aorta to NA ($10^{-5}$ mol/L) in the presence of endothelium (E+), in the absence of endothelium (E−), or after incubation with MB ($10^{-6}$ mol/L). Values are the mean ± SEM. $P < .01$: a *v* b in controls and x *v* y in vitamin E–treated diabetic rats.**

*Morphology of Aorta*

Electron microscopic examination of the aortic media from control rats showed a regular disposition of smooth muscle cells between the elastic laminae within a homogenous interstitial matrix (Fig 4A). Endothelial cells in aortae from control rats were in close contact with the underlying vessel wall and were smooth and uniform (Fig 4B). Intracytoplasmic organization of collagen fibers disappeared from the aortic media of untreated diabetic rats. Smooth muscle cell degeneration together with cell proliferation in aortae was observed in untreated diabetic rats. Internal elastic laminae appeared more developed. The interstitial matrix appeared with large amounts of cell debris (eg, myofibrils, degenerative vesicles, and fibrin crystals; Fig 4C). Adherent platelets and infused granulocytes in the subendothelial region were also observed in aorta from untreated diabetic rats (Fig 4C). Intimal surfaces of aortae from untreated diabetic rats were irregular and showed typical abnormalities. In endothelial cell–free regions of vessels, some subendothelial changes were observed (Fig 4D). Aortae from untreated diabetic rats showed local cytoplasmic edema together with a large number of microvesicles or lysosomes in the cytoplasm of endothelial cells (Fig 4E). Vitamin E treatment of diabetic rats reduced the smooth muscle cell lesions and cell degeneration in aortic media. Collagen fibers were regular (Fig 4F). Endothelial cells of aortae from vitamin E–treated rats and control rats were of approximately similar structure. The intimal surface appeared slightly smoother, with fewer defects. A decrease in the number of microvesicles or lysosomes was observed in endothelial cell cytoplasm. However, lipid drops were still present (Fig 4G).

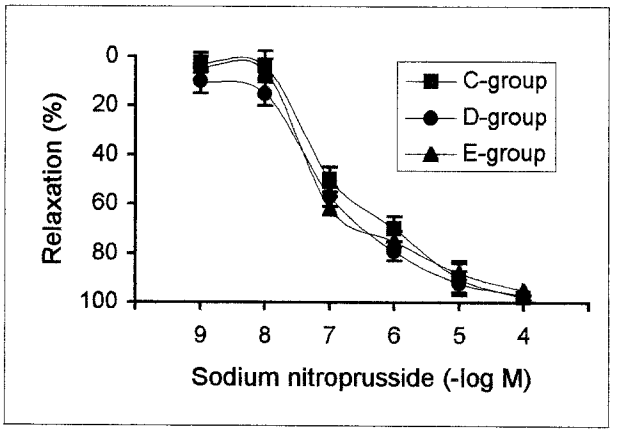


**Fig 3. Concentration-response curves for SNP in aortae with endothelium from control (C group), untreated diabetic (D group), or vitamin E–treated diabetic (E group) rats.**

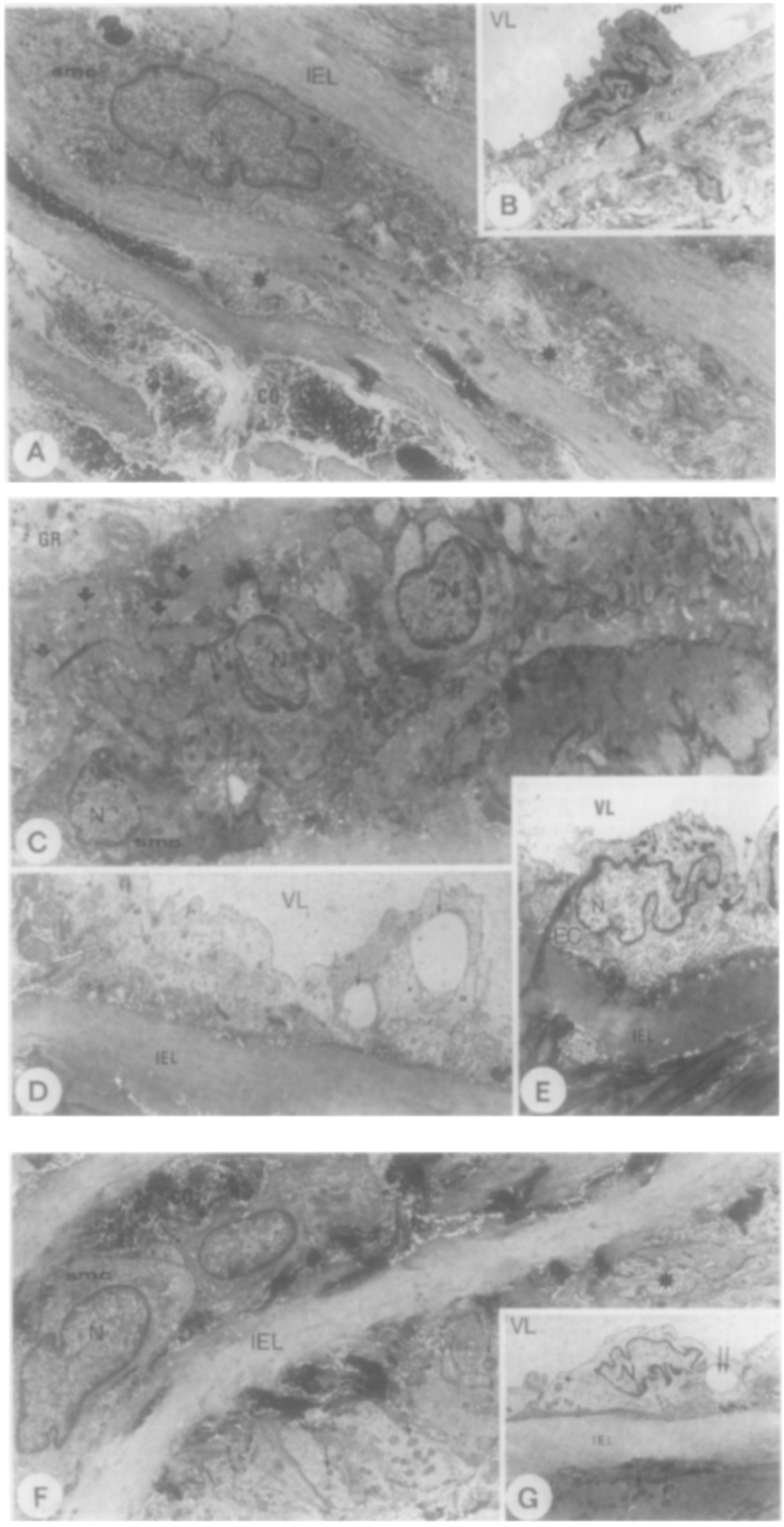


**Fig 4. Transmission electron microscopic characteristics of thoracic aortae. (A) Aspect of tunica media of control aorta. A portion of a smooth muscle cell (smc) can be seen, with a normal nucleus (N) and cytoplasm. Smooth muscle cell layers are separated by regular internal elastic laminae (IEL) that have fine extensions into the interstitial matrix (*). Interstitial matrix contains scattered collagen fibers (CO) (original magnification ×5,400). (B) Representative example of aortic endothelial cell from control rat contains a normal array of organelles (er, endoplasmic reticulum) (×4,500). (C) Irregular profile in smooth muscle cell cytoplasm of aorta from untreated diabetic rat can be seen. IEL has lost its integrity (➧) by infiltration of a granulocyte (GR) from the intimal surface. Intracytoplasmic organization of collagen fibers disappeared. Degenerative vesicles and fibrin crystals (↓) exist in place of tunica media (×5,400). (D) Aortic intima of untreated diabetic rats shows irregular profile together with lipid drops (↓) and subendothelial changes (**) (×5,400). (E) Aortic endothelial cell of untreated diabetic rat shows several cytoplasmic blebs (↓↓). Note the extracellular junction (➧) between endothelial cells (×5,400). (F) In aortic media of vitamin E–treated diabetic rat, smooth muscle cells have a normal appearance without a large defect, but degenerative vesicles (↓) are still present. Collagen fibers and interstitial matrix (*) appear to be regular (×5,400). (G) An aspect of an aortic endothelial cell from vitamin E–treated diabetic rat. Endothelium is relatively smooth, without large defects. A decrease in the number of cytoplasmic blebs and a lipid drop are present (↓↓) (VL, vessel lumen) (×4,500). Uranyl acetate and lead citrate staining.**

## DISCUSSION

The results of the present study show that aortae from 8-week STZ-diabetic rats are more responsive to the contractile effects of both NA and KCl than aortae from corresponding controls. The pharmacological data of the present study confirm and extend our previous observations[28] and are consistent with most previous reports on NA contraction[29-31] and KCl contraction.[32] Among the mechanisms that could have been involved in the enhanced vascular smooth muscle responsiveness in STZ-diabetes are altered phospholipase C and/or G-protein activity

associated with the α-agonist–mediated signal transduction system,[6] enhanced DAG-PKC activity,[15,33] increased calcium influx,[6,29,30] and deficient endothelial activity.[28]

Contraction of the rat aorta to NA can be divided from the mechanical point of view into fast and slow components.[26] The slow component reflects $Ca^{2+}$ influx through receptor-operated channels, and the fast component is caused by release of NA-sensitive intracellular $Ca^{2+}$ stores.[26,30] In the present study, removal of the endothelium or incubation with MB resulted in a significant increase in the slow response of aorta to NA, whereas the fast response remained unaltered in control rats. This observation suggests that only the slow component of NA-induced contraction is suppressed by intact endothelium. In agreement with this, it has been reported that spontaneously released EDRF/NO is a functional antagonist of only the tonic phase of NA-induced contractions by reducing the stimulated influx of extracellular $Ca^{2+}$.[33-35] The magnitude of the slow response to NA in rings pretreated with MB or without endothelium was not significantly different in control rats. This observation indicates that the suppressing effect of aortic endothelium on NA-induced contraction occurs mostly through the release of EDRF/NO rather than other endogen vasodilators, since MB selectively inhibits the vasodilator effect of EDRF by a reduction in cGMP levels through inhibition of soluble guanylate cyclase.[36,37] MB has also been shown to inhibit the synthesis and release of EDRF.[38] In contrast to the findings from control rats, either removal of the endothelium or incubation with MB had no effect on the slow response of aorta to NA in untreated diabetic rats, showing that there is a great deficiency in the suppressing effect of endothelium on the slow response of aorta from untreated diabetic rats to NA. Indeed, a significant reduction in PER values obtained from untreated diabetic rats confirms the presence of endothelial dysfunction in these animals. Consistent with the present findings, both spontaneous production of EDRF and basal levels of cGMP in aorta have been previously demonstrated to decrease in diabetic rats.[39,40] On the other hand, the pharmacological evidence obtained in this study indicates that endothelial deficiency alone is not sufficient to explain the increased responsiveness of aorta to NA in STZ-diabetic rats. The observation that the slow response of aorta with endothelium was significantly higher in untreated diabetic rats than that observed with aorta without endothelium in control rats suggests that intracellular $Ca^{2+}$ availability for the contraction of vascular smooth muscle is increased in STZ-diabetes, and that in addition to endothelial deficiency, this also contributes to the hyperreactivity of the contractile response to NA in diabetic rats. Furthermore, the maximal contraction and sensitivity of aorta to KCl in the absence or presence of endothelium were found to be significantly greater in untreated diabetic rats compared with corresponding controls. It is well known that KCl depolarizes the membrane of smooth muscle cells and is followed by the opening of the voltage-dependent calcium channels and an influx of $Ca^{2+}$ through these channels.[40] Thus, the findings obtained with KCl in diabetic aorta that suggest the existence of an increase in the availability of intracellular $Ca^{2+}$ due to an elevated influx of extracellular $Ca^{2+}$ also support the results obtained with NA in diabetic rats. On the other hand, it is evident from our previous experiments[28] and from the present study that the increased responsiveness of aorta to NA was not due to an alteration in the sensitivity of tissue to this agonist. In addition, because both EDRF and SNP produce vasodilatation by a similar mechanism and SNP relaxation was unaltered by STZ-diabetes, the increased responsiveness of diabetic aorta does not appear to be a consequence of a low responsiveness of vascular smooth muscle cells to normal levels of EDRF/NO.

In a recent study, the increased DAG-PKC activity in hyperglycemic rat aorta was discussed as a result of oxidative stress.[15] Similarly, Chang et al[6] showed that oxidative stress–induced alterations in both voltage-dependent and receptor-operated calcium channel activities were responsible for the increased contractile responses to NA and KCl of aorta in STZ-diabetic rats. In fact, in people with diabetes, any proteins exposed to elevated glucose concentrations undergo increased glycation (nonenzymatic glycosylation).[41] This observation provided the basis for an attractive hypothesis to explain the development of diabetic complications,[42] ie, increased glycation causes structural alterations that in turn lead to functional abnormalities of protein. For example, the process could affect the action of enzymes such as PKC[43,44] or the affinity of receptors for their ligands.[43,44] Furthermore, it was suggested that increased glycation may predispose proteins to oxidative damage,[41] which again could impair function. The mechanisms by which increased glycation of lipoproteins, whether in plasma or in vessel wall, contributes to accelerated development of vascular disease in diabetic patients have been well discussed.[41] It has been demonstrated that advanced glycosylation products quench NO in vitro and in vivo and mediate defective endothelium-dependent vasodilatation in experimental diabetes.[45] Indeed, autoxidation of glucose,[41] lipid peroxidation,[41] and oxidation of glycated proteins[41-43] may lead to an increased release of oxygen-derived radicals and thereby exert an increased oxidative stress on the vasculature in diabetes. The reactive oxygen species have been shown to cause membrane damage and an increase in degradation of EDRF.[5,6] The reduction of EDRF/NO activity has been suggested to promote smooth muscle cell proliferation[3,4] and vasoconstriction.[6,29] It was shown in this study that both plasma triglyceride concentrations and TBA reactivity levels of plasma and of aortae were higher in untreated diabetic rats than in controls. Increased circulating levels of low-density lipoprotein (LDL) and oxidized LDL are believed to have a key role in the inactivation of EDRF associated with diabetes[5,6] both directly and as a result of the release of toxic by-products of oxidation.[46-48] In light of these findings, the possibility of increased degradation of EDRF appears to be a more favorable explanation for the inhibited endothelial response observed in the present study. On the other hand, the ultrastructural examination performed in this study shows that normal tissue organization is disturbed in aortic preparations from STZ-diabetic rats. Although reduced synthesis and/or release of EDRF or inhibition of EDRF action cannot be excluded by the results of this study, the disruption of endothelium and loss of endothelial cells observed in several regions of aortae can account for the reduction of the endothelial response.

It is apparent from the results of the present study that

treatment of diabetic rats with vitamin E partially prevented the increased responsiveness of aorta to NA and KCl, and also inhibited the enhanced fast and slow components of the NA response. The exact mechanism(s) responsible for the promoting effect of vitamin E on the vascular reactivity of STZ-diabetic rats in the present study is not known; however, it seems to be the most likely link with its antioxidant and/or lipid-lowering properties. It was observed in this study that plasma triglyceride concentrations and TBA reactivity levels were lower in vitamin E–treated diabetic rats than in untreated diabetic rats. The inhibitory effects of vitamin E on increased triglycerides or the propagated peroxidation of unsaturated fatty acids have been demonstrated by previous studies in STZ-diabetic rats[18,49,50] and in diabetic patients.[51,52] Vitamin E is a major antioxidant in LDL and is among the first antioxidants to be consumed during LDL oxidation.[8,9,46] In the present study, a deficiency in the inhibitory effect of endothelium on NA-induced contractions in STZ-diabetic rats was shown to be prevented by vitamin E treatment. Moreover, in parallel with the functional protection, aorta structure was also preserved by this vitamin. This is consistent with recent findings by Keegan et al[22] showing that a 1% vitamin E diet prevents the defective endothelium-dependent relaxation in 2-month STZ-diabetic rats. On the other hand, because the inhibitory effect of vitamin E on the proliferation of a variety of cells, including endothelial cells and smooth muscle cells, has been demonstrated,[53] the possibility that the treatment allows normal growth and turnover of vascular cells in diabetic rats should not be excluded from the beneficial effects of vitamin E on the vasculature. In the vitamin E–treated group, the endothelium appears slightly smoother without large defects. On the other hand, vitamin E treatment has recently been shown to inhibit the hyperglycemia-induced activation of PKC in SZT-diabetic rats,[15] which may partly account for the preventive effect of this vitamin on the abnormal contractility observed in our study.

In conclusion, the present findings show that an increase in vascular reactivity together with a deficiency in endothelial function occurs in aortae from STZ-diabetic rats, whereas the general ability of smooth muscle to relax is unaffected. The results also demonstrate that there is a positive correlation between the development of abnormal vessel reactivity and high levels of triglycerides and lipid peroxides in STZ-diabetic rats. Furthermore, the triglyceride-and/or lipid peroxidation–lowering effect of vitamin E treatment associates with the prevention of development of abnormalities in the function and structure of aortae in STZ-diabetic rats. Therefore, we suggest that vitamin E is an eligible candidate for protection of the vascular wall against oxidative injury associated with diabetes mellitus.

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