ACE Inhibitors Improve Endothelial Function in Type 1 Diabetic Patients With Normal Arterial Pressure and Microalbuminuria

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OBJECTIVE — The purpose of this study was to test whether a short-course treatment with ACE inhibitors may restore endothelium-dependent and/or -independent vasodilation in the femoral artery of microalbuminuric patients with type 1 diabetes and normal arterial pressure.

RESEARCH DESIGN AND METHODS — We studied nine normotensive microalbuminuric type 1 diabetic patients and two groups of control subjects matched for femoral artery diameter to type 1 diabetic patients after placebo (control group A, $n = 17$) and ACE inhibitor (control group B, $n = 18$) treatment, respectively. The patients were enrolled in a double-blind cross-over study with a 1-week trial of either placebo, captopril (25 mg t.i.d.), or enalapril (10 mg/day) in randomized order to ascertain whether short-term ACE inhibition obtained with (captopril) or without (enalapril) a sulfhydryl donor molecule ameliorates vessel wall function. Endothelium-mediated flow-dependent vasodilation and endothelium-independent vasodilation were evaluated in the right common femoral artery by echo Doppler.

RESULTS — Both captopril and enalapril normalized (control group B 22.9 ± 3.2% per 8 min) endothelium-dependent response (19.6 ± 7.5 and 18.0 ± 5.3 vs. −10.4 ± 4.1% per 8 min, $P < 0.01$, for both captopril and enalapril versus placebo, respectively) in the type 1 diabetic patients. Captopril (28.4 ± 3.5 vs. 17.1 ± 3.5% per 5 min during placebo, $P < 0.05$) but not enalapril (20.1 ± 3.0 vs. 31.7 ± 2.8% per 5 min, $P < 0.05$ for enalapril versus control group B, and NS for captopril vs. control group B) ameliorated endothelium-independent vasodilation in type 1 diabetic patients.

CONCLUSIONS — ACE inhibition improves endothelium-dependent vasodilation in the femoral artery of normotensive microalbuminuric type 1 diabetic patients. Captopril also ameliorates endothelium-independent vasodilation, possibly through its sulfhydryl donor properties. These results may be of pathophysiological relevance to prevent cardiovascular complications in these patients.

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Abbreviations: ANOVA, analysis of variance; CV, coefficient of variation; ECG, electrocardiogram; GTN, glyceryl trinitrate.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Cardiovascular complications and end-stage renal failure are the main causes of morbidity and death in patients with type 1 diabetes. A crucial role in the pathogenesis of diabetic vascular complications is ascribed to endothelial abnormalities (1). The fine balancing between endothelium-derived relaxing factors and prostacyclin, with their vasodilatory and antiaggregatory effects, and endothelin and other cyclooxygenase products (prostaglandins and thromboxane A_2), with their vasoconstrictive and proaggregatory effects, is fundamental in preventing atherogenesis (2).

Endothelium- and NO-dependent vasodilation in human forearm microcirculation has been abnormal in type 1 diabetic patients in many studies (3–7) but not in others (8,9). Most studies, with only one exception (5), demonstrated a reduced endothelium-independent vasodilatory response to NO donors (3,8,9). This defect was attributed to the presence of advanced glycosylation end products and/or increased generation of the superoxide anion in type 1 diabetic patients, both of which are known to effectively antagonize the biological activity of NO. Furthermore, basal NO-dependent vasodilatory tone has been found to be reduced in type 1 diabetic patients (10).

In an earlier study, we measured femoral artery dilation in response to an increase in blood flow in the femoral vascular bed in type 1 diabetic patients (11). This biological response is mediated by the endothelium through a transduction system independent of the one activated by the muscarinic receptors and in humans is entirely NO mediated (12). We found that type 1 diabetic patients had a reduced response to the flow- and shear stress-dependent stimulus. Even more strikingly, type 1 diabetic patients with microalbuminuria displayed a paradoxical vasoconstriction. In addition, the endothelium-independent response to an NO donor was blunted in the type 1 diabetic patients (11). Similar findings have been documented by other laboratories (7,13,14), although Enderle et al. (15) reported pre-

served endothelial function in type 1 diabetic patients.

Thus, type 1 diabetic patients, and in particular those with microalbuminuria, display a triad of functional vascular defects: a reduction in the basal NO-dependent vasodilatory tone (10), an endothelium-dependent defect in NO-mediated vasodilation (3–7,11,13,14), and a blunted endothelium-independent vasodilatory response to NO donors (3,8,9,11,13,14).

The gradient of vascular functional defect between nonmicroalbuminuric and microalbuminuric patients is not unexpected (11,14). Indeed, in epidemiological surveys, microalbuminuria predicted the development of cardiovascular complications in type 1 diabetic patients (16). Thus, this parameter may be considered an indicator of early vascular damage in which functional lesions may be detected, such as an impairment in vasorelaxant capacity (3–7,11,13,14).

ACE inhibitors can improve endothelium-dependent vasodilation both in vitro and in vivo (17–19). Among the ACE inhibitors, captopril, because of its sulfhydryl moiety, also may amplify the effects of NO donor substances by protecting newly released NO against the scavenging action of superoxide anion and/or free radicals (20). These effects seem to be independent of the blood pressure–lowering action of these agents (21). Thus, ACE inhibitors as a class may positively influence endothelium-dependent vascular dysfunction, and captopril in particular may be especially effective in restoring a decreased vascular response to NO donor substances.

Two recent articles examined the effect of ACE inhibition on endothelium-dependent vasodilation in type 1 diabetic patients. O'Driscoll et al. (5) reported that enalaprilat, in an open trial design, may normalize acetylcholine-induced forearm vasodilation in type 1 diabetic patients without microalbuminuria and with a normal response to an NO donor. Mullen et al. (22), in a randomized double-blind parallel-group study of 91 patients, reported no effects of 6-month enalapril treatment on flow-dependent and -independent vasodilation assessed in the brachial arteries of normoalbuminuric type 1 diabetic subjects. Therefore, no data are available regarding microalbuminuric type 1 diabetic patients.

The aim of this study was to investigate whether short-term treatment with two different ACE inhibitors (a sulfhydryl donor, captopril, and a nonsulfhydryl donor, enalapril) can improve endothelium-dependent and -independent vasodilation in type 1 diabetic patients with microalbuminuria and normal arterial pressure.

RESEARCH DESIGN AND METHODS

Subjects

Nine (3 men, 6 women; mean age 33 ± 3 years, BMI 22.4 ± 1.2 kg/m^2, HbA_{1c} 7.9 ± 0.6%, fasting plasma glucose 12.3 ± 1.7 mmol/l, insulin dose 37.1 ± 4.8 U/day) microalbuminuric type 1 diabetic patients were enrolled in a randomized double-blind cross-over study. Duration of diabetes was 13 ± 4 years. All subjects had persistent microalbuminuria (i.e., an albumin excretion rate between 20 and 200 µg/min on at least two determinations during a 6-month period). None of the subjects had hypertension, hyperlipidemia, obesity, or clinical evidence of macroangiopathy, which was further documented by normal echo-Doppler scans of carotid and femoral arteries and normal electrocardiograms (ECGs). On retinal fundoscopic examination, six patients had no signs of diabetic retinopathy, two had background retinopathy, and one had proliferative retinopathy.

To provide a reference set of normal values for the femoral artery vasoactive responses, we selected two groups of healthy subjects, control groups A (n = 17) and B (n = 18), who were matched according to basal femoral artery diameter to type 1 diabetic patients after placebo and to type 1 diabetic patients after ACE inhibitors, respectively. The control groups were similar to the type 1 diabetic patients regarding sex (control group A, 10 women, 7 men; control group B, 13 women, 5 men) and BMI (control A, 23.2 ±0.9 kg/m^2; control group B, 22.0 ± 0.4 kg/m^2) but were somewhat younger (control A, 23.9 ± 0.8 years; control group B, 23.6 ± 0.5 years; $P < 0.01$ for both versus type 1 diabetic patients). Both control groups were studied under the same experimental conditions described below.

The purpose, nature, and potential risks of the study were explained to all subjects, and informed written consent was obtained before their participation. The protocol was reviewed and approved by the Institutional Review Board at Verona City Hospital.

Study protocol

Each type 1 diabetic patient underwent a 1-week trial of either placebo, captopril (75 mg/day), or enalapril (10 mg/day) in randomized order. Each treatment was followed by a 2-week washout period. Basal biochemical parameters were assessed at enrollment after an overnight fast. At the end of each treatment, humoral parameters and noninvasive evaluation of femoral artery reactivity were assessed 120 min after a mid-morning snack and the accompanying dose of regular insulin. Femoral artery reactivity was assessed in the healthy subjects under the same experimental conditions as in the type 1 diabetic patients, except the control subjects were not administered insulin.

The vascular tests were performed at a controlled room temperature of 22 ± 1°C after a 15-min rest in supine position. Blood pressure and heart rate were measured every minute by an oscillometric recorder (DYNAMAP model 845; Critikon, Tampa, FL) (23) positioned on the left arm. High-resolution echo-Doppler (Sonos 1000; Hewlett-Packard, Milano, Italy) with a 7.5-MHz linear vascular probe that has an axial resolution of 0.1 mm was used to measure flow velocity and arterial diameter in the right common femoral artery at a fixed distance from the femoral bifurcation. These measurements were performed basally during distal postischemic hyperemia and after administration of glyceryl trinitrate (GTN). Arterial diameter, which was considered to be the distance between the M lines of the proximal and distal wall (i.e., the interface between the tunica media and adventitia, which is easier to detect than the surface of the endothelial layer), was measured on a two-dimensional ultrasound image of a longitudinal section by using ultrasonic calipers at the end-diastolic phase of the cardiac cycle (identified by an accompanying ECG) to avoid errors of evaluation due to arterial wall distensibility. A Doppler-derived flow measurement was calculated by using a pulsed-wave Doppler signal at a 60° angle to the vessel wall with the range gate (1 mm) in the center of the artery and multiplying its velocity by time integral by the heart rate and the arterial cross-sectional area. The peak increment in blood flow velocity after distal hyperemia was considered to be the best surrogate measure of the biological stimulus that induces an endothelium-dependent vasodilation. The reproducibility of these methods was evaluated in five control subjects in whom 12 consecutive measurements were performed by the same observer during a 90-min period. Under these conditions, the coefficients of variation [CVs] were 1.8 ± 0.8 and 14 ± 3% for femoral diameter and blood flow velocity, respectively.

Table 1—*Biochemical and hemodynamic parameters after placebo, captopril, and enalapril treatment in type 1 diabetic microalbuminuric patients*

	Placebo	Captopril	Enalapril
Plasma glucose (mmol/l)	8.6 ± 2.4	10.3 ± 0.9	8.8 ± 1.3
Fructosamine (μmol/l)	443 ± 23	421 ± 18	423 ± 24
Albumin excretion rate (μg/min)	46.9 ± 9.8	48.6 ± 7.2	40.7 ± 9.7
Systolic blood pressure (mmHg)	126 ± 2.3	124 ± 3.1	124 ± 2.7
Diastolic blood pressure (mmHg)	75.6 ± 1.9	72.4 ± 2.8	70.9 ± 1.9*
Mean arterial pressure (mmHg)	88.3 ± 2.3	86.3 ± 3.0	84.2 ± 2.3
Heart rate (bpm)	73.3 ± 6.2	70.6 ± 5.8	75.6 ± 5.2

Data are means ± SEM. $n = 9$. *$P < 0.05$ vs. placebo treatment.

Distal ischemia was obtained by applying a mercury sphygmomanometer cuff around the calf and inflating it to suprasystolic pressure for 8 min. During distal hyperemia, the increase in flow velocity and the changes in the diameter of the femoral artery were measured 0.5, 2, 4, and 8 min after the end of ischemia. Endothelium-independent vasodilation was evaluated 3.5 and 5 min after administration of GTN (400 μg sublingual).

Global quantitative indexes of flow (endothelium)-dependent and endothelium-independent vasodilation were obtained by calculating the area under the curve of change in vessel diameter as a function of time expressed both as absolute values and as the percentage of change over the baseline vessel diameter. Note that the face values of these areas are several times greater than the changes in large conduit artery diameters during similar tests commonly reported in the literature. This is because the latter ones most often refer to a single point assessment or to a time-averaged response (13). Approximate comparisons with reports from other laboratories can be easily made by dividing our results of endothelium-dependent and -independent vasodilation by 8 and 5, respectively.

Because an impaired response of endothelium-independent vasodilation was anticipated in our microalbuminuric type 1 diabetic patients (11,14), the ratios of flow (endothelium)-dependent vasodilation to endothelium-independent vasodilation were also calculated, both with the absolute and the percentage values, in an attempt to factor out the role of the postendothelium steps in determining the femoral artery response to an acceleration in blood flow (shear stress), which has been suggested by others (13). These ratios are referred to in this article as the "absolute endothelial index" when computed as the ratio of the absolute values and "percent endothelial index" when computed as the ratio of the percentage values.

The minimum detectable absolute difference in femoral vasodilation in a paired study was calculated with the following formula:

$$\delta = s_*(t_{\alpha(2),m} + t_{\beta(1),m})/(n^{1/2})$$

where δ is the minimum detectable difference, s is the standard deviation of the variable within subjects, $t_{\beta(1),m}$ and $t_{\alpha(2),m}$ are the t values corresponding to a 90% $(1 - \beta)$ chance of detecting a 0.05 $[\alpha(2)]$ level of significance with m degrees of freedom, and n is the number of observations (24). We calculated the value of s in five healthy subjects not included in the control group of this study who were known to have a wide range of endothelium-dependent vasodilation from normal to subnormal responses. They were studied on 2 different days, at least 2 weeks apart, according to the above protocol. In these five individuals, endothelium-dependent vasodilation was 25.4 ± 7.8% per 8 min on one occasion and 20.4 ± 5.6% per 8 min on the other occasion (NS). The average within-subject standard deviation of the endothelial response (s) was 4.1 ± 1.85% per 8 min (within-subject CV 20.1 ± 5.9%). From these values, we calculated that this study had a 90% chance to detect an absolute difference between treatments in femoral vasodilation of at least 5.69% per 8 min at a P level of 0.05.

Statistical analysis

Data are means ± SEM. One-way analysis of variance (ANOVA) was carried out to detect differences between control subjects and type 1 diabetic patients. One-way ANOVA for repeated measures was performed to assess the treatment effect within type 1 diabetic patients. When the group or the treatment effect was statistically significant, individual comparisons were carried out by Fisher's protected least significant difference test. Statistical significance was achieved at $P < 0.05$ (24).

RESULTS — Basal blood pressure was minimally decreased during ACE inhibitor treatment (Table 1) in the type 1 diabetic patients, with a statistically significant effect obtained with enalapril for diastolic values ($P = 0.047$). Control A had lower arterial blood pressure (systolic pressure 118 ± 2.2 mmHg, diastolic pressure 69 ± 1.9 mmHg) than placebo-treated type 1 diabetic patients ($P < 0.05$), whereas, after ACE inhibition, no statistically significant difference was detected between patients and control group B (systolic pressure 117 ± 2.7 mmHg, diastolic pressure 67 ± 1.5 mmHg). Basal heart rate was not affected by either captopril or enalapril (Table 1). Metabolic control as assessed by fructosamine, plasma glucose during the hemodynamic test, and microalbuminuria was also similar during the three treatments (Table 1).

ACE inhibition in the patients was associated at baseline with a decrease in femoral artery diameter (8.28 ± 0.29, 7.93 ± 0.18, and 7.96 ±0.17 mm for placebo, captopril, and enalapril groups, respectively; $P < 0.05$ for both captopril and enalapril versus placebo). Femoral artery diameter of control A (8.28 ± 0.21 mm) and control group B (7.92 ± 0.21 mm) was similar to type 1 diabetic patients after placebo and ACE inhibitor treatment, respectively. Distal postischemic hyperemia elicited similar increases in blood flow velocity in the type 1 diabetic patients after the three treatments (placebo 20.0 ± 3.0, captopril 20.5 ± 2.0, enalapril 25.8 ± 1.4 cm/s, NS), thereby exposing femoral endothelium to comparable stimuli in all occasions. In control subjects, postischemic acceleration of blood flow (control A 14.4 ± 1.7 cm/s, control group B 16.9 ± 3.7 cm/s) was not statistically different from type 1 diabetic patients treated with either placebo or ACE inhibitors.

Changes in femoral artery diameter at each time point during the vascular tests are reported in Table 2. Endothelium-dependent femoral artery response and the endothelial indexes were significantly reduced in the placebo-treated type 1 diabetic patients compared with control A ($P < 0.01$ for all groups) (Figs. 1 and 2). Endothelium-independent vasodilation was somewhat but not significantly ($P = 0.11$) (Fig. 3)

Table 2—*Changes in femoral artery diameter versus baseline after endothelium-dependent and -independent stimulation*

	Control group A	Control group B	Type 1 diabetic groups		
			Placebo	Captopril	Enalapril
Endothelium-dependent stimulation/min					
Δ[0.5] (mm)	+0.32 ± 0.04	+0.38 ± 0.07	−0.12 ± 0.06*	+0.35 ± 0.14†	+0.30 ± 0.06†
Δ[2.0] (mm)	+0.32 ± 0.04	+0.38 ± 0.04	−0.13 ± 0.07*	+0.35 ± 0.13†	+0.23 ± 0.09†
Δ[4.0] (mm)	+0.20 ± 0.04	+0.22 ± 0.03	−0.09 ± 0.05*	+0.17 ± 0.07†	+0.16 ± 0.08†
Δ[8.0] (mm)	+0.02 ± 0.01	+0.03 ± 0.02	−0.15 ± 0.07*	+0.02 ± 0.04	+0.13 ± 0.04
Δ[0.5] (%)	+3.88 ± 0.54	+4.94 ± 0.94	−1.45 ± 0.78*	+4.51 ± 1.86†	+3.78 ± 0.75†
Δ[2.0] (%)	+3.78 ± 0.40	+4.85 ± 0.56	−1.48 ± 0.87*	+4.48 ± 1.7†	+2.92 ± 1.24†
Δ[4.0] (%)	+2.38 ± 0.43	+2.88 ± 0.47	−1.0 ± 0.58*	+2.21 ± 0.91†	+1.99 ± 1.0†
Δ[8.0] (%)	+0.23 ± 0.17	+0.42 ± 0.30	−1.67 ± 0.82*	+0.31 ± 0.48	+1.58 ± 0.51†
Endothelium-independent stimulation/min					
Δ[3.5] (mm)	+0.61 ± 0.07	+0.74 ± 0.05	+0.44 ± 0.09	+0.74 ± 0.09†	+0.50 ± 0.08‡
Δ[5.0] (mm)	+0.53 ± 0.05	+0.81 ± 0.07	+0.40 ± 0.09	+0.57 ± 0.11	+0.53 ± 0.11‡
Δ[3.5] (%)	+7.52 ± 0.90	+9.53 ± 0.83	+5.37 ± 0.97	+9.26 ± 1.13†	+6.14 ± 0.94‡
Δ[5.0] (%)	+6.56 ± 0.74	+10.5 ± 1.1	+4.95 ± 1.04	+7.02 ± 1.32	+6.42 ± 1.26‡

Data are means ± SEM. Changes (Δ) in femoral artery diameter versus baseline after endothelium-dependent and -independent (400 µg sublingual GTN) stimulation in control groups A (n = 17, matched to placebo-treated type 1 diabetic patients) and B (n = 18, matched to captopril- and enalapril-treated type 1 diabetic patients) and in placebo-, captopril-, and enalapril-treated type 1 diabetic patients (n = 9) are shown. *$P < 0.01$ or less vs. control group A at the same time point; †$P < 0.05$ or less vs. placebo treatment at the same time point; ‡$P < 0.05$ or less vs. control group B at the same time point.

reduced in the placebo-treated type 1 diabetic patients compared with control A.

The paradoxical vasoconstriction observed in the placebo-treated type 1 diabetic patients (−10.4 ± 4.1% per 8 min, $P < 0.05$ versus zero and $P < 0.01$ versus control A) subsequent to the flow-dependent endothelial stimulus was reverted to a significant vasodilation by both ACE inhibitory agents (19.6 ± 7.1 and 18.0 ± 5.3% per 8 min for captopril and enalapril, respectively, $P < 0.01$ versus placebo) with no significant differences between captopril and enalapril. With both ACE inhibitors, endothelium-dependent vasodilation was not significantly different in the patients compared with control group B (22.9 ± 3.2% per 8 min). A similar pattern is observed when the results are expressed as absolute values (Fig. 1).

In the type 1 diabetic patients, after GTN administration, captopril (28.4 ± 3.5% per 5 min) but not enalapril (20.1 ± 3.0% per 5 min) treatment was associated with a better endothelium-independent femoral artery dilation than placebo (17.1 ± 2.8% per 5 min, $P < 0.05$ versus captopril and NS versus enalapril). Furthermore, captopril but not enalapril treatment achieved an endothelium-independent vasodilation similar to control group B (31.7 ± 2.8% per 5 min, NS versus captopril, $P < 0.05$ versus enalapril). A similar pattern is observed when the results are expressed as absolute values (Fig. 3).

In the patients, both the percent (Table 3) and absolute (Fig. 2) endothelial indexes were significantly improved by both captopril ($P < 0.01$) and enalapril ($P < 0.01$) treatment versus the values observed during the treatment with placebo and restored back to the control values (Table 3 and Fig. 2).

CONCLUSIONS — Pharmacological ACE inhibition has become increasingly popular among physicians treating microalbuminuric patients with type 1 diabetes because it can slow down or even prevent the deterioration of renal function (25). Microalbuminuria is also an important indicator of cardiovascular risk both in this class of patients and in the general population, and there is increasing evidence that,

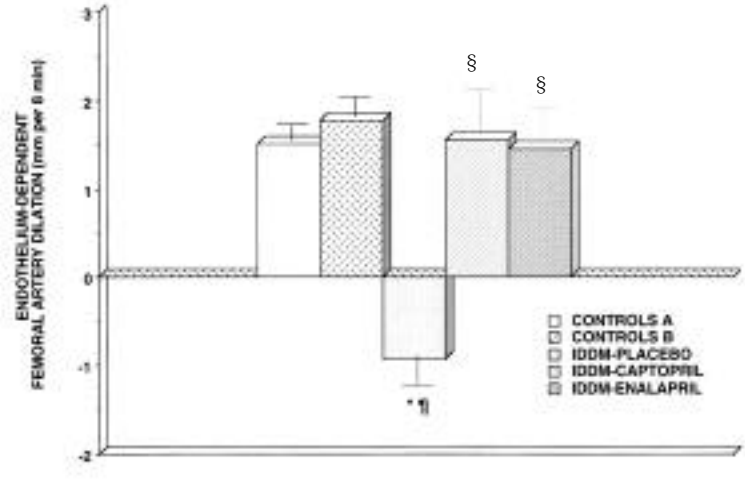

**Figure 1—*Endothelium (flow)-dependent femoral artery vasodilation (absolute values) in control subjects* A (n = *17) and* B (n = *18) and placebo-, captopril-, and enalapril-treated type 1 diabetic patients* (n = *9).* *P < *0.05 vs. zero;* ¶P < *0.01 vs. control subjects;* §P < *0.01 vs. placebo treatment.*

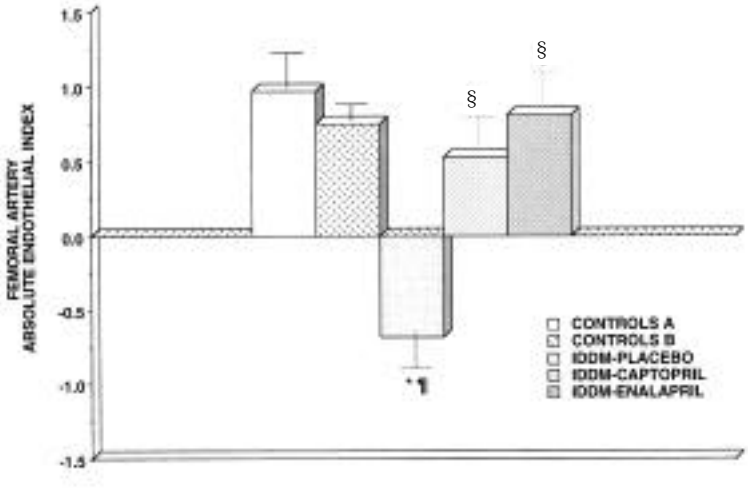

Figure 2—*Femoral artery endothelial index (ratio of endothelium-dependent to endothelium-inde - pendent vasodilation in absolute values) in control subjects A* (n = *17) and B* (n = *18) placebo-, capto - pril-, and enalapril-treated type 1 diabetic patients* (n = *9*). *P < *0.05 vs. zero;* ¶P < *0.01 vs. control subjects;* §P < *0.01 vs. placebo treatment.*

at least in selected groups of nondiabetic patients, treatment with ACE inhibitors can exert a significant cardiovascular protective effect (21,26).

In this article, we report the results of a double-blind placebo-controlled trial that tested the effects of a short course (1 week) of two ACE inhibitors on endothelium-dependent and -independent arterial dysfunction in patients with type 1 diabetes and microalbuminuria. Importantly, our patients were normotensive to rule out possible independent effects of altered blood pressure on endothelium-dependent biological responses (27).

Captopril and enalapril did not significantly affect either plasma glucose during the test or metabolic control in our patients (Table 1). This point is particularly relevant in that glycemic control, as reflected by HbA_{1c} levels, is negatively correlated to acetylcholine-induced vasodilation in the forearm of type 1 diabetic subjects (4). In these patients, duration of the disease, LDL cholesterol (13), and microalbuminuria (11) but not HbA_{1c} levels (28) are good indicators of endothelial dysfunction in large conduit vessels. We did not measure insulin levels during the hemodynamic studies in our patients. Because insulin can affect endothelial responses, at least in normal individuals, the possible role of different insulin levels in our studies cannot be ruled out, although the randomized placebo-controlled design should have prevented any systematic influence of uncontrolled factors.

In addition, microalbuminuria was unaffected by both ACE inhibitors, probably because of the short duration of treatment (Table 1). Both agents caused a small (although statistically significant in enalapril-treated patients) decrease in diastolic blood pressure, whereas systolic pressure and heart rate were unchanged (Table 1). Thus, all the effects exerted by captopril and enalapril on endothelial function were achieved despite barely detectable changes in blood pressure and albumin excretion rate.

The main finding of this study is that both captopril and enalapril reverted the paradoxical constriction of the femoral artery after an NO-releasing stimulus into a vasodilatory response (Fig. 1). This correction of femoral artery endothelial dysfunction is most likely to be secondary to common mechanisms of action of the two drugs that affect either the endothelial production of NO, NO stability, or NO effectiveness. Several possible explanations for this finding exist.

Both captopril and enalapril may blunt the local synthesis of angiotensin II and inhibit bradykinin catabolism, thereby increasing vasodilation by multiple mechanisms (29,30), including NO production and effectiveness (31,32).

A decrease in local angiotensin II production is particularly desirable in patients with type 1 diabetes in whom both the renin-ACE system and vascular sensitivity to angiotensin II are abnormally elevated (33,34). Additional possible effects of angiotensin II are stimulation of preproendothelin gene transcription (35), facilitation of endothelin-1 release (35), enhanced endothelial production of PGH_2 (prostaglandin H) (36), and modulation of the tonic superoxide anion ($\cdot O_2^-$) release from the vessel wall (37).

Thus, we speculate that ACE inhibition improved endothelium-dependent vasodilation in this study, probably by resetting the balance between angiotensin II and bradykinin actions in the vessel wall.

In the type 1 diabetic patients, captopril but not enalapril treatment induced an endothelium-independent response of the femoral artery to GTN (an NO donor) that

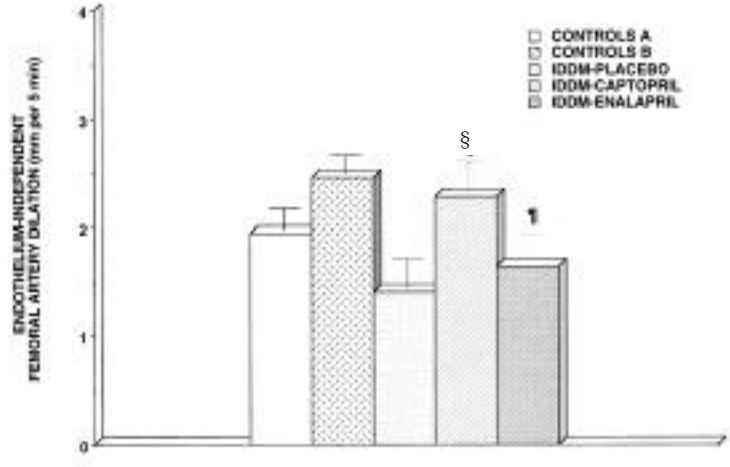

Figure 3—*Endothelium-independent (GTN-induced) femoral artery vasodilation (absolute values) in control subjects A* (n = *17) and B* (n = *18) and placebo-, captopril-, and enalapril-treated type 1 diabetic patients* (n = *9*). ¶P < *0.01 vs. control subjects;* §P < *0.01 vs. placebo treatment.*

Table 3—*Absolute endothelial index (ratio of endothelium-dependent to -independent femoral artery vasodilation in absolute values) and percent endothelial index (ratio of endothelium-dependent to -independent femoral artery vasodilation in percentages)*

	Control group A	Control group B	Type 1 diabetic groups		
			Placebo	Captopril	Enalapril
Absolute endothelial index	0.973 ± 0.23	0.752 ± 0.11	−0.683 ± 0.24*	0.533 ± 0.24†	0.815 ± 0.26†
Percent endothelial index	0.978 ± 0.23	0.760 ± 0.11	−0.660 ± 0.24*	0.538 ± 0.24†	0.835 ± 0.27†

Data are means ± SEM. Control group A (n = 17) was matched to placebo-treated type 1 diabetic patients; control group B (n = 18) was matched to captopril- and enalapril-treated type 1 diabetic patients. n = 9 for placebo-, captopril-, and enalapril-treated type 1 diabetic patients. *$P < 0.01$ vs. control group A; †$P < 0.01$ vs. placebo treatment.

was almost superimposable to the one observed in control group B (Fig. 3). This apparently agent-specific effect may be due to the sulfhydryl donor property of captopril, possibly through antagonism of thiol groups against free radicals. This interpretation, albeit speculative, agrees with several studies that showed that, in type 1 diabetes, there is a widespread increase in free radical production (38).

Both captopril and enalapril reverted the endothelial index in the type 1 diabetic patients back to normal (Table 2 and Fig. 2), further confirming that ACE inhibition in our patients may have restored a normal endothelial release of NO in response to shear stress (Fig. 1). The interpretation of the endothelial index, however, must be viewed with caution because it relies on the unproved assumption that the two vasodilatory stimuli (shear stress and GTN) work on the same part of the NO dose response curve.

The results of this study agree with those of O'Driscoll et al. (5), who reported that enalaprilat, in an open trial design, could normalize acetylcholine-induced forearm vasodilation in type 1 diabetic patients. Our study, however, differs from O'Driscoll et al.'s in several ways, including selection of the patients (our patients were microalbuminuric, whereas O'Driscoll et al.'s patients were not), study design (our study was double-blind and placebo controlled), stimulus applied (shear stress versus a muscarinic agonist), vessels under investigation (large conduit artery versus resistance vessels), and timing of vascular function assessment (we studied the patients before a significant change in blood pressure could be documented, whereas O'Driscoll et al. performed the study after enalapril had caused a fall in blood pressure).

Mullen et al. (22), in a randomized double-blind parallel-group study, reported no effects of 6-month enalapril treatment on flow-dependent and -independent vasodilation in type 1 diabetic patients assessed in the brachial artery. However, of the 91 patients studied by Mullen et al., 90 were normoalbuminuric, whereas we selected only microalbuminuric patients, who display a much more severe degree of vascular dysfunction (11). Finally, glucose control was worse in Mullen et al.'s study (average HbA_{1c} 10.2%) than in our patients, a factor that may blunt the effects of ACE inhibition on endothelial dysfunction.

We therefore believe that our findings are not repetitious of either O'Driscoll et al.'s (5) or Mullen et al.'s (22) observations in that they are relevant to a large conduit vessel prone to clinical atherosclerosis in a group of type 1 diabetic patients who are burdened with a much higher risk of developing cardiovascular complications (39).

In summary, a short course of ACE inhibitor therapy ameliorates the defect in the endothelium (NO)-dependent response of the femoral artery (a target vessel for atherosclerosis) to a physiological stimulus (e.g., shear stress) in microalbuminuric type 1 diabetic patients. Furthermore, captopril, possibly through its sulfhydryl donor properties, may also improve the endothelium-independent response of the femoral artery to a NO donor. Whether ACE-inhibition can protect these patients against macrovascular complications remains to be determined.

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