

Effect of Enalapril on Endothelial Function in Young Insulin-Dependent Diabetic Patients: A Randomized, Double-Blind Study

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Objectives. We sought to determine whether 6 months of treatment with the angiotensin-converting enzyme (ACE) inhibitor enalapril can improve conduit artery endothelial function in young subjects with insulin-dependent diabetes mellitus (IDDM).

Background. Endothelial dysfunction is an early event in atherogenesis and has been demonstrated in young subjects with IDDM. ACE inhibitors have been shown to enhance conduit artery endothelial function in animal experiments and in patients with established coronary atherosclerosis, although their effect in IDDM is not known.

Methods. Ninety-one subjects (mean age 30.9 years, range 18 to 44) with stable IDDM but no clinical evidence of vascular disease were randomized to receive enalapril (20 mg once daily) (46 subjects) or placebo (45 subjects) in a randomized, double-blind, parallel-group study. Brachial artery flow-mediated dilation (FMD), an endothelium-dependent stimulus, and response to glyceryl trinitrate (GTN), which acts directly on vascular smooth

muscle, were assessed noninvasively by means of high resolution external vascular ultrasound at baseline and after 12 and 24 weeks of treatment.

Results. FMD was inversely correlated with total cholesterol ($r = 0.22$, $p = 0.041$) but not with any diabetic variables. Treatment with enalapril had no significant effect on FMD ($p = 0.67$) or response to the endothelial-independent dilator GTN ($p = 0.45$).

Conclusions. These data suggest that impairment of endothelial-dependent dilation in young subjects with IDDM is not improved by treatment with the ACE inhibitor enalapril. This lack of improvement may reflect the complex nature of vascular disease in IDDM, which can affect both endothelial and smooth muscle function.

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In addition to the clinical consequences of microangiopathy, subjects with insulin-dependent diabetes mellitus (IDDM) have increased morbidity and mortality from coronary, cerebral and peripheral atherosclerosis (1,2). By the age of 55 years, the cumulative mortality from coronary artery disease (CAD) can be as high as 30% (1), and furthermore, the progression of atherosclerosis is greater and the results of revascularization less effective (3,4). Endothelial dysfunction is thought to contribute to the pathogenesis of both microangiopathy and macrovascular disease in diabetes (5-7) and has been demonstrated in patients with IDDM from as early as the

second decade of life (8-10). Reduced bioavailability of the "antiatherogenic" molecule nitric oxide (NO) results in impaired endothelium-dependent dilation (11), enhanced platelet aggregation, leukocyte/endothelial cell interactions and smooth muscle cell proliferation and migration (11-15).

The renin-angiotensin system and its inhibition may influence endothelial function through a number of different pathways (16). Angiotensin-II may promote macrophage activation and smooth muscle cell proliferation, both early events in atherogenesis (17,18). Free radical generation results in inactivation of NO, enhanced lipid peroxidation and cellular injury (19,20). Angiotensin-converting enzyme (ACE) inhibitors may have a beneficial effect on endothelial function by reducing angiotensin-II levels (16) and also by inhibiting bradykinin degradation (21). ACE inhibitors have been shown to improve large-vessel endothelial function in both animal models of hypercholesterolaemia (22,23) and clinical studies of subjects with established CAD (24). In IDDM, benefits on the progression of renal disease have been reported (25,26), but the effects on large-vessel endothelial function have not been studied. We therefore investigated the effect of the ACE inhibitor enalapril

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Abbreviations and Acronyms

ACE	=	angiotensin converting enzyme
CAD	=	coronary artery disease
CI	=	confidence interval
ECG	=	electrocardiogram, electrocardiographic
FMD	=	flow-mediated dilation
GTN	=	glyceryl trinitrate
HbA1c	=	glycosylated hemoglobin
IDDM	=	insulin-dependent diabetes mellitus
LDL	=	low density lipoprotein
NO	=	nitric oxide

on conduit artery endothelial function in young subjects with IDDM to determine whether improvements of potential clinical significance could be achieved from an early stage in the natural history of arterial disease.

Methods

Subjects. Subjects with IDDM >2 years in duration were selected from a diabetic clinic in London if they fulfilled the following criteria: 18 to 45 years old, nonsmoker (had not smoked >2 years, with a total exposure <1 pack-year), rest supine blood pressure <140/90 mmHg, no clinical evidence of large-vessel atherosclerosis and were taking no vasoactive medication. One hundred eleven subjects were evaluated with a detailed clinical history, physical examination and 12-lead electrocardiogram (ECG). To characterize subjects for the presence of microvascular disease, two or more timed overnight urine collections were made to measure urinary albumin excretion at recruitment and again on completion of the study. After a 4-week screening phase during which all subjects received placebo medication, 91 of the 111 subjects with stable diabetic control (glycosylated hemoglobin [HbA1c] <14%) and satisfactory compliance with placebo were entered into the randomized phase of the study. Reasons for nonrandomization were high HbA1c in 6 patients, poor cooperation or withdrawal of consent in 12 and adverse experience in 2.

Study design. The study was designed as a randomized, double-blind, parallel-group study. During the first 3 weeks, therapy in each subject was titrated from a starting dose of 5 mg of enalapril or placebo equivalent to a dose of 20 mg or the maximal dose tolerated. Endothelial function was assessed noninvasively, at baseline and after 12 and 24 weeks of treatment by means of high resolution external ultrasound, as previously described (8,27). Subjects were instructed to continue their normal diabetic diet and insulin regime for the duration of the study. No attempt was made to study subjects under conditions of euglycemia. To do so would have been logistically difficult and, moreover, would have introduced the potential confounding influences of a glucose and insulin infusion on endothelial function. Blood samples were taken at each visit for full blood count, biochemical analysis and HbA1c measurement (Rapid EP system, Helena Laboratories). Fast-

ing total cholesterol and plasma triglycerides were measured at recruitment and on completion of the study by means of the cholesterol C-system high performance CHOD-PAP and GPO-PAP high performance enzymatic colorimetric test, respectively (Boehringer-Mannheim GmbH, Diagnostica). High density lipoprotein (HDL) cholesterol was measured after precipitation of apoprotein B-containing lipoproteins and low density lipoprotein (LDL) cholesterol calculated according to the Friedwald formula (28). The study was approved by the local research ethics committee, and all subjects gave written informed consent.

Assessment of endothelial function. Subjects rested supine for at least 10 min before the first scan and remained supine throughout the procedure. The right brachial artery was imaged in the longitudinal section 2 to 10 cm above the elbow using a standard 7-MHz linear array transducer supported by a flexible stereotactic clamp and an Accuson 128XP/10 ultrasound system. The center of the artery was identified when the clearest image of anterior and posterior arterial wall layers was seen. Depth and gain settings were set to optimize the lumen/arterial wall interface, and the transmit (focus) zone was set to the depth of the anterior wall because of the greater difficulty in evaluating this interface. Machine-operating variables were not altered for the duration of the study. The image was magnified using a resolution box function, and rest blood flow was estimated using pulsed wave Doppler with the cursor set at 70° to the longitudinal axis of the artery and the range gate (1.5 mm) in the center of the artery. A segment of the artery was selected for analysis, and 5 s of radiofrequency signal from this segment was routed to an A-mode tracking device (Ingenious Systems, The Netherlands). After careful placement of volume sample cursors at the vessel wall interfaces, vessel wall movement was tracked and end-diastolic diameter determined on a beat by beat basis with a spatial resolution of 50 μm.

Once a stable and clear image was achieved, the transducer position was fixed, and both the arm and transducer remained in the same position throughout the study. Hard copy images of the brachial artery were taken and notes made of the transducer and arm position, enabling precise reproduction of conditions and measurement of the same segment of artery at subsequent visits. Rest brachial artery internal diameter was measured at end-diastole for consecutive beats over a 5-s interval, and the mean internal diameter was calculated. Reactive hyperemia was induced by inflation to 300 mm Hg of a pneumatic tourniquet placed around the forearm at a site distal to that being scanned and its rapid release after 4.5 min. The increase in brachial artery flow over 15 s after cuff release was determined using pulsed wave Doppler, as described above. Brachial artery diameter was measured 55 to 65 s after release of the tourniquet, and flow-mediated dilation (FMD) was expressed as the percent increase in diameter in response to reactive hyperemia. After 10 to 15 min rest to allow vessel recovery, a further rest scan was recorded. Sublingual glyceryl trinitrate (GTN) (400 μg) was administered, and the response to this endothelium-independent dilator was assessed after 3 min. Brachial artery flow-mediated dilation (FMD) may be

blocked by specific antagonists of NO synthase (29), and measures of FMD using this technique correlate with invasive assessments of coronary endothelial function and atherosclerosis (30). Hemodynamic and ECG parameters were monitored throughout the study.

Data analysis. All scans were recorded on super VHS videotape. Printouts of the A-mode signal and cursor placement were made for each scan. Scans were checked for technical errors by two independent observers (M.J.M., A.E.D.) who had no knowledge of the origin of the scan. Scans in which the image at subsequent studies was not replicated, a satisfactory distensibility waveform was not achieved or cursor placement was incorrect were excluded from the final analysis. Rest volumetric flow was calculated for each study by multiplying the velocity-time integral corrected for angle by the heart rate and vessel cross-sectional area. This method may lead to overestimation of blood flow although inaccuracies are consistent, allowing comparison between visits and between subjects. Reactive hyperemia was calculated as the ratio of maximal flow (determined from the pulse wave Doppler signal and heart rate) during the first 15 s after tourniquet release to that at baseline and was expressed as the percent increase in flow.

Statistical analysis. Descriptive data are expressed as mean value \pm SD. Subjects who were withdrawn after randomization were included in the analyses up to and including the time of withdrawal. Changes in the measures of vascular function were assessed by two-way repeated measure analysis of variance and adjusted for multiple comparisons using the Bonferroni test. Simple univariate correlation coefficients were determined between the measures of baseline endothelial-dependent and -independent dilation and subject demographic (age, gender) and diabetic characteristics (duration of diabetes, insulin dose, HbA1c, blood pressure) and lipoprotein subfractions [triglycerides; total, LDL and HDL cholesterol, lipoprotein(a)]. These variables were then entered into a stepwise multiple regression analysis to explore factors that may determine rest blood flow and vascular responses to reactive hyperemia and GTN. A similar stepwise multiple regression model was constructed, with change in FMD over the duration of the study as the dependent variable and the addition of treatment group as an independent variable. Statistical significance was inferred at $p < 0.05$.

Results

Baseline subject characteristics. Age, levels of lipid subfractions, blood pressure, length of time since diagnosis of diabetes, total daily insulin dose and HbA1c as a measure of overall diabetic control were comparable in both groups (Table 1). Only one subject had microalbuminuria (20 to 200 $\mu\text{g}/\text{min}$), and none had macroproteinuria.

Of the 91 subjects randomized (46 to enalapril, 45 to placebo), 39 taking enalapril and 43 placebo completed the study. Five subjects (four with enalapril, one with placebo) withdrew because of adverse clinical experiences, and four subjects (three with enalapril, one with placebo) withdrew

Table 1. Baseline Characteristics and Vascular Reactivity

	Enalapril Group (n = 46)	Placebo Group (n = 45)
Male	65.2%	57.8%
Age (yr)	29.6 (6.3)	32.1 (6.5)
Duration of IDDM (yr)	12.9 (7.8)	12.5 (8.3)
HbA1c (%)	10.2 (1.6)	10.3 (1.8)
Total daily insulin dose (IU)	57.0 (17.26)	51.6 (20.8)
SBP (mm Hg)	116.9 (10.3)	118.6 (8.7)
DBP (mm Hg)	73.7 (8.8)	74.6 (7.5)
Total cholesterol (mmol/liter)	5.06 (0.89)	5.05 (1.01)
LDL cholesterol (mmol/liter)	3.08 (0.80)	2.95 (0.92)
HDL cholesterol (mmol/liter)	1.53 (0.33)	1.63 (0.38)
Triglycerides (mmol/liter)	0.95 (0.60)	0.95 (0.43)
Lipoprotein(a) (mmol/liter)*	8.65 (0.05-101.80)	8.20 (0.05-103.40)
Vessel diameter (mm)	3.59 (0.57)	3.55 (0.61)
Rest blood flow (ml/min)*	30 (12-119)	24 (8-167)
Reactive hyperemia (%)*	311 (125-653)	354 (125-653)
FMD (%)	1.60 (2.38)	2.31 (2.68)
GTN-mediated dilation (%)	17.47 (8.04)	18.34 (7.56)

*Skewed data expressed as geometric mean (range). Data presented are mean value (SD), unless otherwise indicated. DBP = diastolic blood pressure; FMD = flow-mediated dilation; GTN = glyceryl trinitrate; HbA1c = glycosylated hemoglobin; HDL = high density lipoprotein; IDDM = insulin-dependent diabetes mellitus; LDL = low density lipoprotein; SBP = systolic blood pressure.

because of protocol violations or for administrative reasons. Forty-three of 46 enalapril and 44 of 45 placebo group patients achieved titration to the maximal dose (20 mg or placebo equivalent), although the dose was subsequently reduced in seven enalapril and six placebo group subjects. The treatments were well tolerated, and compliance determined by tablet count was $>80\%$ in all but three subjects. Twenty-two enalapril and 20 placebo group subjects had a clinically adverse event during the study. Six enalapril and one placebo group subject complained of cough, and six subjects (4 taking enalapril, 2 placebo) had symptoms of headache or dizziness that were thought to be related to the study medication. However, no serious events thought to be related to the study medication were recorded. Fifteen scans from 12 subjects (9 taking enalapril, 3 placebo) were withdrawn for technical reasons, as described above.

There were no significant changes in diabetic control, insulin requirement or lipid subfractions over the course of the study (Table 1). Enalapril caused a mean decrease of 3.4 ± 10.0 mm Hg of diastolic blood pressure (95% confidence interval [CI] -6.7 to -0.1), but systolic blood pressure remained unchanged, as did both parameters in the placebo group.

Vascular function. At baseline, rest vessel size, brachial artery blood flow, degree of reactive hyperemia, FMD and dilation in response to GTN were comparable in the enalapril and placebo groups (Table 1). On univariate analysis, there was an inverse relation between total cholesterol levels and FMD ($r = -0.22$, $p = 0.041$). On stepwise multiple regression analysis, total cholesterol at baseline, but no other lipoprotein subfractions or diabetic characteristics, was independently

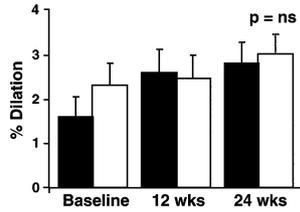


Figure 1. FMD (mean ± SEM). There was no significant change in endothelial-dependent dilation either within or between the treatment groups (two-way repeated measures analysis of variance adjusted for multiple comparisons by the Bonferroni test). **Solid bars** = enalapril; **open bars** = placebo.

correlated with FMD (beta -0.62 , $p = 0.041$). Dilation in response to GTN was independently related to gender (beta 5.24 , $p = 0.0026$), rest blood flow velocity (beta -66.5 , $p = 0.015$) and total cholesterol level (beta -2.26 , $p = 0.0076$). In a separate stepwise multiple regression analysis, rest blood flow correlated with vessel diameter (beta 24.3 , $p < 0.0001$) and inversely with total cholesterol level (beta -8.39 , $p = 0.0076$) but not with any diabetic variables or blood pressure. This finding may indicate an abnormality of basal tone in distal resistance arterioles related to cholesterol levels.

Enalapril had no effect on rest vessel size, brachial artery blood flow or the degree of reactive hyperemia induced, suggesting that it did not cause significant vasodilation in this normotensive cohort. FMD for the whole group at baseline was $2.17 \pm 2.69\%$, considerably reduced compared with that in nondiabetic subjects of similar age and risk factor profile previously studied in our laboratory (8) and confirming the presence of endothelial dysfunction. In subjects treated with enalapril, mean FMD increased from $1.60 \pm 2.38\%$ at baseline to $2.55 \pm 2.68\%$ and $2.80 \pm 2.94\%$ at 12 and 24 weeks, respectively. In the placebo group, FMD also increased from $2.31 \pm 2.68\%$ to $2.58 \pm 2.19\%$ and $3.02 \pm 2.64\%$, respectively (Fig. 1). However, the increase in FMD over the duration of the study did not reach statistical significance ($p = 0.27$, 95% CI -0.19 to 0.67), and there was no significant difference between responses of the enalapril and placebo groups ($p = 0.671$, 95% CI -0.34 to 0.52). Response to the endothelium-independent dilator GTN did not change significantly in either group over the duration of the study ($p = 0.25$, 95% CI -0.54 to 2.07) or in response to enalapril ($p = 0.44$, 95% CI -1.80 to 0.81) (Fig. 2).

Figure 2. GTN-mediated dilation (mean ± SEM). Enalapril (**solid bars**) had no significant effect on endothelial-independent dilation (two-way repeated measures analysis of variance adjusted for multiple comparisons by the Bonferroni test). **Open bars** = placebo.

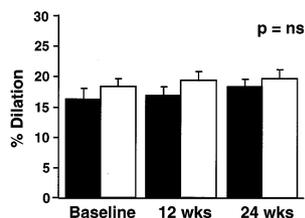


Table 2. Determinants of Change in Flow-Mediated Dilation*

	Regression Coefficient	p Value
Baseline FMD	-0.68	< 0.0001
Vessel diameter	-1.54	0.003
Duration of IDDM	-0.097	0.004
SBP	-0.07	0.029

*Multiple $r = 0.48$; $F = 2.42$. Abbreviations as in Table 1.

Multiple regression analysis was performed to determine whether any factors in the subjects' baseline profile or treatment group influenced change in FMD over the study period. Improvement in FMD was related to baseline FMD, vessel size, duration of diabetes and systolic blood pressure but not with dilation in response to GTN, age, HbA1c level, LDL cholesterol level or treatment group (Table 2).

Discussion

The present study confirmed our previous finding (8) of marked abnormalities of conduit artery physiology in young, clinically well subjects with IDDM, despite the absence of microvascular disease. Vascular dysfunction was related to total and LDL cholesterol levels but not to measures of recent metabolic control of diabetes. Despite experimental evidence of favorable effects of ACE inhibition in early atherosclerosis, we were unable to demonstrate an improvement in conduit artery FMD during 6 months of treatment with the ACE inhibitor enalapril.

ACE inhibitors and vascular function. ACE inhibitors potentially have a number of beneficial effects on vascular structure and function, mediated either by the direct inhibition of the promitogenic and proinflammatory effects of angiotensin-II, or by enhanced bioavailability of the "anti-atherogenic" molecule NO (31). Increased NO activity may result from attenuation of angiotensin-II-mediated production of superoxide (19,32), which can inactivate NO (20), or through inhibition of bradykinin degradation, a potent physiologic stimulus for NO release (21,33). Experimental evidence suggests that ACE inhibition may reduce subendothelial accumulation of macrophages (34,35), inhibit smooth muscle cell growth and enhance endothelial cell repair factors, which may be important in the early stages of atherogenesis (17,18,36). In hypercholesterolemic rabbits, ACE inhibition has been shown (22,23) to enhance endothelial-dependent dilation and to reduce the development of atherosclerosis independently of its blood pressure-lowering effects. In clinical trials, a similar benefit on coronary endothelial function has been shown (24) with quinapril in nondiabetic patients with CAD, but no benefit on atherosclerosis or clinical outcome has been demonstrated.

In the current study, we have investigated a group of young subjects with IDDM because they are known to be at risk for developing premature atherosclerosis. We chose subjects without clinical vascular disease or other risk factors for athero-

sclerosis to examine the impact of ACE inhibition on early abnormalities of endothelial function in IDDM. The majority of our subjects (90 of 91) did not have microalbuminuria, which is known to increase the risk of atherosclerosis and to benefit from ACE inhibitor therapy (1,25,26).

The lack of an improvement after enalapril treatment compared with placebo may have a number of explanations. There was a trend toward an increase in FMD in both the enalapril and placebo groups. The reason for the placebo group improvement may reflect subtle changes in subject behavior during the study that we were unable to detect or control, and it remains possible that a significant result would have been achieved with a greater number of subjects or a longer study period. We chose to study subjects over 6 months because this period has been shown (24) to result in significant improvement in coronary endothelial function in patients with CAD treated with quinapril when, intuitively, disease is likely to be more advanced and resistant to therapy.

A second factor may be the greater importance of the renin-angiotensin system in established atherosclerosis. Recent evidence (37) suggests that expression of ACE inhibitors in conduit artery endothelium may be low in the absence of atherosclerotic plaque, and the beneficial effects of ACE inhibition seen in animal and human studies may therefore reflect a greater impact on more advanced atherosclerotic disease. Furthermore, the vasoprotective effects of ACE inhibitors may depend on their ability to inhibit tissue-bound as opposed to circulating ACE (38). The potency of individual ACE inhibitors may be influenced by their pharmacologic properties, such as lipid solubility, affinity for ACE in vascular tissues and their clearance (39). Our findings with enalapril cannot therefore be extrapolated to other ACE inhibitors.

Endothelial function in IDDM. The complex nature of vascular injury in IDDM, which affects both endothelial and smooth muscle cell function, may be less responsive to intervention than that seen in young subjects with early risk factors, such as hypercholesterolemia (40). This may influence the ability to detect changes in endothelial function using this noninvasive technique. Endothelial dysfunction in IDDM may result from enhanced effects of general cardiovascular risk factors, including hypertension and a shift to an atherogenic lipoprotein profile (41), as well as factors specific to IDDM affecting both NO production and breakdown. Oxidative stress is increased, and levels of the antioxidant vitamins E and C, catalase and superoxide dismutase may all be reduced in diabetic tissues (42). Nonenzymatic glycosylation of proteins may lead to the formation of advanced glycosylation end products that may also inactivate NO, initiate lipid peroxidation and cause endothelial damage after uptake by specific cellular receptors (43-45). NO production may also be decreased as a result of defects of NO synthase or decreased availability of L-arginine, the substrate for NO synthesis (46,47). Noninvasive assessment of endothelial function relies on the contrast between endothelial-dependent and -independent vasodilation, so that endothelial-derived NO activity (29) can be determined. In addition to abnormalities of

FMD, impaired response to the smooth muscle dilator GTN has been demonstrated in diabetic patients (8) and may limit the sensitivity of our methodology to detect subtle improvements in endothelial function in response to intervention.

Our previous work (8) has indicated that the abnormalities of conduit vascular function in IDDM were related to duration rather than control of diabetes. The present study extends these findings by demonstrating that for the whole cohort, duration of diabetes was also related to improvement in FMD over 6 months, although we cannot ascribe this result to a treatment effect of enalapril. This finding is in accord with clinical studies in which tight metabolic control has had disappointing results on the progression of large-vessel atherosclerosis in IDDM (48). Despite our inclusion of a relatively young cohort of subjects with IDDM, earlier intervention may be required to achieve greatest benefit.

Although the renin-angiotensin system may have a role in the pathogenesis of vascular disease, the complex nature of this process in IDDM may be resistant to individual strategies aimed at restoring vascular function. Thus, in addition to specific therapies, including ACE inhibition, an approach that targets reduction of modifiable risk factors, such as hypercholesterolemia, known to be associated with early endothelial damage, or aimed at enhancing endothelial production of NO may be required to limit the clinical impact of large-vessel atherosclerosis in IDDM.

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